ECOSPHERE

Inferring phytoplankton community composition with a fatty acid mixing model

U. Strandberg,[1],† S. J. Taipale,[2] M. Hiltunen,[1] A. W. E. Galloway,[1,3,5] M. T. Brett,[4] and P. Kankaala[1]

[1]Department of Biology, University of Eastern Finland, Joensuu FI-80101 Finland
[2]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä FI-40014 Finland
[3]Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm SE-10691 Sweden
[4]Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington 98195-2700 USA

Abstract. The taxon specificity of fatty acid composition in algal classes suggests that fatty acids could be used as chemotaxonomic markers for phytoplankton composition. The applicability of phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton composition was evaluated by using a Bayesian fatty acid-based mixing model. Fatty acid profiles from monocultures of chlorophytes, cyanobacteria, diatoms, euglenoids, dinoflagellates, raphidophyte, cryptophytes and chrysophytes were used as a reference library to infer phytoplankton community composition in five moderately humic, large boreal lakes in three different seasons (spring, summer and fall). The phytoplankton community composition was also estimated from microscopic counts. Both methods identified diatoms and cryptophytes as the major phytoplankton groups in the study lakes throughout the sampling period, together accounting for 54–63% of the phytoplankton. In addition, both methods revealed that the proportion of chlorophytes and cyanobacteria was lowest in the spring and increased towards the summer and fall, while dinoflagellates peaked in the spring. The proportion of euglenoids and raphidophytes was less than 8% of the phytoplankton biomass throughout the sampling period. The model estimated significantly lower proportions of chrysophytes in the seston than indicated by microscopic analyses. This is probably because the reference library for chrysophytes included too few taxa. Our results show that a fatty acid-based mixing model approach is a promising tool for estimating the phytoplankton community composition, while also providing information on the nutritional quality of the seston for consumers. Both the quantity and the quality of seston as a food source for zooplankton were high in the spring; total phytoplankton biomass was ~56 µg C L^{-1}, and the physiologically important polyunsaturated fatty acids 20:5n-3 and 22:6n-3 comprised ~22% of fatty acids.

Key words: algae; Bayesian mixing model; chemotaxonomic marker; FASTAR; food quality; freshwater phytoplankton; polyunsaturated fatty acid; seasonality; zooplankton.

[5] Present address: School of the Environment, Washington State University, Pullman, Washington 99164-2812 USA.
† E-mail: ursula.strandberg@uef.fi

INTRODUCTION

Phytoplankton have a fundamental role as primary producers in aquatic food webs and are susceptible to both natural and anthropogenic environmental changes. The composition and abundance of freshwater phytoplankton is highly dynamic, and varies with temperature, irradi-

ance, nutrient availability and pollution (Moss et al. 2003, Thackeray et al. 2008). Phytoplankton are major suppliers of energy and nutrients to upper trophic level consumers, and due to strong trophic coupling, phytoplankton biomass and community composition may also be controlled by grazers (Moss et al. 2003). Knowledge on the composition and abundance of phytoplankton is commonly used for evaluating the ecological status of both marine and freshwater ecosystems and is essential for the understanding the structure and dynamics of aquatic ecosystems (McCormick and Cairns 1994, Birk et al. 2012). Algal produced polyunsaturated fatty acids (PUFA) are considered essential to zooplankton and fish, because most animals cannot produce these molecules de novo (Tocher 2003). The fatty acid composition of algae is largely phylogenetically determined (Dalsgaard et al. 2003, Taipale et al. 2013), thus the availability of biochemically important PUFA in the aquatic food web is strongly influenced by the phytoplankton community composition.

Microscopic analysis is the traditional tool for studies of phytoplankton community composition (Utermöhl 1958). Although this approach provides detailed information on the species composition, microscopy requires extensive training and is quite time consuming. Moreover, detailed information on species composition is rarely needed and therefore data are usually summed to represent higher order taxonomic groups or alternatively classified according to the occurrence and abundance in specific environmental conditions, so called functional classification (Reynolds et al. 2002). New techniques for evaluating phytoplankton community composition include biochemical markers (Dijkman and Kromkamp 2006), flow-based imaging cytometry (Steinberg et al. 2012) and application of spectral fluorescence signatures (Gregor and Maršálek 2004, Schlüter et al. 2006). Spectral imaging techniques are based on the different fluorescence of photosynthetic pigments, but colored dissolved organic matter (CDOM) can interfere with the taxonomic discrimination of phytoplankton (MacIntyre et al. 2010). In chemotaxonomic approaches, the taxon specificity of the biomarkers is essential. The most common chemotaxonomic markers for phytoplankton are photosynthetic pigments (Gregor and Maršálek

2004, Schlüter et al. 2006), followed by fatty acids (Léveillé et al. 1997, Reuss and Poulsen 2002, Boschker et al. 2005).

Only a few fatty acids are truly taxon specific and the applicability of single fatty acid biomarkers is therefore limited. For example, the common diatom biomarker eicosapentaenoic acid (20:5n-3, EPA) has been documented to poorly indicate diatom blooms that are dominated by *Cyclotella* (Sushchik et al. 2004), suggesting that EPA alone is not a definitive biomarker for diatoms as a group because it is also very prevalent in other phytoplankton, e.g., cryptophytes (Taipale et al. 2013). However, the entire fatty acid composition of diatoms, including *Cyclotella*, is identifiable and separable from other phytoplankton groups; specifically diatoms are characterized by very high proportions of 16:1n-7 and very low levels of 18 carbon chain (C_{18}) PUFAs (Taipale et al. 2013). Proportional differences of the entire fatty acid profile between taxa are large and multivariate approaches indicate that phytoplankton may be differentiated to class level (Dalsgaard et al. 2003, Taipale et al. 2013). The fatty acid composition of specific algae taxa is to some extent plastic; light conditions, temperature, nutrient conditions, and the age of the algal culture can lead to intraspecific variation in the lipid and fatty acid composition (Reitan et al. 1994, Piepho et al. 2012), but the compositional differences between algal classes are greater than possible intraspecific variation due to varying environmental conditions (Bi et al. 2014). Also in nature changes in phytoplankton community composition are likely to be a more prevalent response to varying environmental conditions than pronounced ecophysiological and biochemical adaptations within algal species.

Phospholipids are major components of cell membranes and phospholipid-derived fatty acids (PLFA) as chemotaxonomic markers are widely used in terrestrial microbial ecology (Zelles 1999, Frostegård et al. 2011). The advantage of using PLFA for chemotaxonomic inference is that phospholipids are rapidly degraded after cell senescence and thus in field samples PLFA are considered to represent living cells. The application of both photosynthetic pigments and PLFA as biomarkers for the evaluation of community composition of estuarine phytoplankton suggested that compared to the pigment analysis, the

larger number of markers in the PLFA analysis provide methodological robustness (Dijkman and Kromkamp 2006). CHEMTAX software (Mackey et al. 1996) is most commonly used for inferring the phytoplankton composition from biochemical markers, but shortcomings related to the selection of biomarker input ratios have been suggested (Van den Meersche et al. 2008). Bayesian based statistics would allow full use of biomarker data as well as make it possible to characterize uncertainty (credibility intervals) for the final estimates (Van den Meersche et al. 2008).

Seston fatty acids are analyzed for various purposes, particularly for evaluating zooplankton diets. The aim of the current study was to evaluate whether fatty acid data could also be used for the evaluation of the phytoplankton composition, and thus provide additional ecological information for e.g., food web studies. A Bayesian based fatty acid mixing model approach (e.g., Galloway et al. 2014a, 2014b) was applied to infer the phytoplankton community composition from seston PLFA. The fatty acid composition of cultured algae (Ahlgren et al. 1992, Taipale et al. 2013) was used as a reference library to infer the phytoplankton composition in moderately humic large boreal lakes. However, this model can be applied to any aquatic ecosystem by adjusting the reference library. Humic lakes were chosen for this study, because high concentrations of CDOM and humic substances have been shown to interfere with e.g., algal pigment fluorescence based techniques (MacIntyre et al. 2010). To evaluate model performance, mixing model estimates of the phytoplankton community composition was also compared to microscopic cell counts.

MATERIAL AND METHODS

Seston samples were collected from large lakes in eastern Finland in the spring (end of May), summer (beginning of August) and fall (end of September) of 2011. The sampled lake basins were Kallavesi, Suvasvesi (two sampling locations), Orivesi (two sampling locations), Paasivesi, and Pyhäselkä (details of the lakes are provided in Appendix A). About 40 L of water from the surface to 4 m was pumped and sieved through a 50 μm screen, from which samples for standard measurements of water chemistry (dissolved organic carbon (DOC), total nitrogen, total phosphorus), as well as samples for chlorophyll a, microscopic counts and analyses of phospholipid-derived fatty acids were collected. The DOC concentration of water (<0.2 μm) was analyzed with a Multi N/C (Analytic Jena) instrument. Total phosphorus, total nitrogen and chlorophyll a concentrations were measured with standard methods (SFS 3026, SFS-EN ISO 11905-1, SFS 5772). About 250 ml of sieved water was preserved in Lugol's solution for microscopic counting, and ~2 L was filtered through a GF/F filter (Whatman) for PLFA analyses. Replicates of the PLFA samples were prepared at each sampling location and time. The filter was immediately placed in a vial containing chloroform/methanol (2:1 by vol). The lipids were extracted from the filter and the polar lipid fraction was obtained via solid phase extraction using silica cartridges (500 mg, Agilent). The cartridges were dehydrated with methanol and preconditioned with chloroform; the sample was introduced in chloroform and thereafter eluted with 10 ml chloroform and 10 ml acetone. The polar lipid fraction was eluted with 10 ml of methanol and evaporated to dryness. The polar lipids were suspended into toluene and transmethylated by adding 1% sulfuric acid in methanol and heating at 60°C for 16 hours. The fatty acid methyl esters (FAME) were extracted twice with n-hexane/diethyl ether (1:1 by volume), evaporated to dryness and suspended into n-hexane.

A reference library of fatty acid profiles of freshwater algae monocultures was used to represent the end-members (sources) in the mixing model. The reference library consisted mostly of literature values (Ahlgren et al. 1992, Taipale et al. 2013), but was supplemented with original samples from various cyanobacteria (Anabaena, Aphanothece, Microcystis, Planktothrix, two strains of Pseudanabaena and Synechococcus) and a dinoflagellate (Peridinium). All of the taxa included in the library are commonly present in the study lakes (Table 1). The cyanobacteria and Peridinium were cultured in either Z8 or WC media using a 14:10 light:dark cycle, in a temperature controlled chamber at 20°C (see Taipale et al. 2013 for details). The total lipids were extracted from freeze-dried algae and transmethylated as described above. To compare

Table 1. The most abundant genera in the lake samples (genera present in the reference library appear in boldface), percentage of algal genera (within different algal groups) that are the same in the reference library and in the lake samples, and the proportion of biomass of those genera in the lake samples that are present in the reference library.

Phytoplankton group	Most abundant genera in lake samples	Percentage of group genera	Percentage of group biomass
Cyanobacteria	**Anabaena**, *Aphanizomenon*, **Planktothrix**	33	43
Cryptophytes	**Cryptomonas, Rhodomonas**	100	100
Dinoflagellates	**Peridinium**, *Glenodinium*	33	30
Chrysophytes	**Mallomonas**, *Pseudopedinella*	20	39
Diatoms	**Asterionella, Aulacoseira, Cyclotella**	33	79
Raphidophytes	**Gonyostomum**	100	100
Euglenoids	**Euglena**, *Trachelomonas*	50	66
Chlorophytes	**Chlamydomonas, Pediastrum**, *Oocyctis, Monoraphidium*	13	39

the differences in the fatty acid composition of total lipids and phospholipids in the cultured algae, phospholipid-derived FAME were also prepared for cultured chlorophytes, diatoms, cryptophytes, chrysophytes and dinoflagellates. The FAME of all phytoplankton cultures were analyzed using a gas chromatograph equipped with mass detector (Shimadzu Ultra) at the University of Jyväskylä (Finland) and the FAME of seston samples were analyzed with a gas chromatograph (Agilent 6890N) with mass spectrometric detection (Agilent 5973N) at the University of Eastern Finland. Both instruments were equipped with an Agilent DB-23 column (30 m × 0.25 mm × 0.15 µm). Identification of FAME was based on mass spectra, as well as reference standards GLC-566C and GLC-68D from Nu Chek-Prep. Further details on the chromatographic and mass spectrometric conditions can be found in Taipale et al. (2013).

Statistical analyses and modeling

The Bayesian fatty acid-based mixing model Fatty Acid Source Tracking Algorithm in R (FASTAR), which is an extension of the stable isotope mixing model MixSIR (Moore and Semmens 2008), was used to infer the phytoplankton community composition from seston fatty acids. The FASTAR model was developed to infer basal resource use of zooplankton and benthic macroinvertebrates (Galloway et al. 2014*a*, 2014*b*). In the current study, the model uses a reference library input matrix consisting of the mean and SD of fatty acids in different algal classes and a matrix of the fatty acid profiles of lake seston samples (mean of replicates) to calculate the

potential proportions of algal classes that best matches the fatty acid profiles found in the field samples.

All algal groups in the reference library included fatty acid data from multiple genera, with the exception of the Raphidophyceae, which only has one known species (*Gonyostomum semen*) in boreal humic lakes. All fatty acids (n = 23) that were found in both the seston samples and in the reference library were included in the FASTAR analysis (Table 2). The phytoplankton composition was estimated for seven stations in five lake basins in three seasons (n = 20, with one missing sample). The FASTAR results are summarized as posterior distributions that describe the probability of each algal group's contribution to the phytoplankton community composition in the field samples. An uninformative prior (i.e., Dirichlet with an alpha = 1) was used, following the original model structure of the stable isotope mixing model MixSIR (Moore and Semmens 2008). The median FASTAR solution was used to compare to the microscopic counts, but the full posterior distributions from each analysis are also reported. The FASTAR algorithm was run in R (R Development Core Team 2014; version 3.0.1). Multidimensional scaling (MDS, based on Euclidean distances) was used to visualize the compositional differences in the seston. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in the seston fatty acid and phytoplankton composition from different seasons and lake basins as fixed and random factors, respectively. The significance of analyses was determined using unrestricted permutation of the raw data

Table 2. Seasonality of seston phospholipid derived fatty acids in moderately humic large lakes (n = 20). Values are expressed as percentage of total phospholipid derived fatty acids in seston (mean with SD in parentheses). Different letters indicate significant difference between seasons (ANOVA followed by Games-Howell, P < 0.05).

Fatty acid	Spring	Summer	Fall
14:0	3.0 (3.3)	5.0 (1.6)	3.4 (2.2)
i-15:0†	0.4 (0.4)	0.9 (0.3)	0.7 (0.3)
ai-15:0†	0.3 (0.3)	0.3 (0.1)	0.5 (0.3)
15:0	0.2 (0.1)	0.4 (0.6)	0.3 (0.2)
i-16:0†	0.1 (0.1)	0.0 (0.1)	0.0 (0.1)
16:0	16.9^A (2.0)	24.3^B (3.9)	19.6^B (5.3)
16:1n-9	0.1^A (0.1)	0.2^{AB} (0.2)	0.4^B (0.2)
16:1n-7	11.9 (4.8)	11.2 (2.1)	10.6 (1.5)
16:1n-5	0.7^A (0.9)	2.1^B (0.7)	1.8^{AB} (0.7)
i-17:0†	0.0 (0.0)	0.0 (0.1)	0.1 (0.1)
ai-17:0†	0.1 (0.2)	0.0 (0.0)	0.0 (0.1)
17:0	0.1 (0.1)	0.3 (0.2)	0.3 (0.2)
16:2n-4	0.3^A (0.2)	0.8^B (0.4)	1.0^B (0.4)
16:3n-4	0.1 (0.2)	0.1 (0.2)	0.5 (0.4)
16:3n-3	0.0^A (0.0)	0.2^B (0.2)	0.4^B (0.3)
16:4n-3	0.1^A (0.1)	0.7^B (0.4)	0.6^B (0.4)
18:0	6.7 (3.8)	7.7 (4.6)	6.7 (4.8)
18:1n-9	1.6^A (0.5)	2.7^B (0.3)	3.5^B (0.7)
18:1n-7	2.1 (1.5)	2.8 (0.8)	4.5 (2.7)
18:2n-6	1.2^A (0.6)	2.4^B (0.6)	2.4^B (0.7)
18:3n-6	0.2 (0.2)	0.3 (0.3)	0.2 (0.2)
18:3n-3 (ALA)	9.1^A (2.4)	12.6^B (1.6)	12.0^{AB} (2.0)
18:4n-3	16.2 (4.7)	14.9 (4.4)	14.0 (2.8)
18:5n-3†	2.8^A (0.7)	0.4^B (0.4)	2.8^{AB} (3.5)
20:0†	0.1^A (0.1)	0.1^{AB} (0.1)	0.0^B (0.0)
20:2n-6†	0.6 (0.5)	0.2 (0.3)	0.5 (0.4)
20:4n-6 (ARA)	1.6 (2.0)	1.3 (1.4)	1.3 (0.6)
20:4n-3	0.0 (0.1)	0.1 (0.1)	0.0 (0.0)
20:5n-3 (EPA)	13.9^A (1.7)	5.5^B (2.3)	8.7^C (1.8)
22:5n-6	1.3 (1.4)	0.7 (0.9)	0.6 (0.4)
22:6n-3 (DHA)	8.2^A (2.6)	1.7^B (1.8)	2.2^B (1.3)
SFA	26.6^A (4.0)	36.9^B (6.8)	29.7^{AB} (9.1)
MUFA	16.4 (6.3)	19.0 (2.2)	20.8 (3.9)
PUFA	55.7 (8.4)	41.8 (6.1)	47.2 (9.6)
Bacterial FA	1.3 (1.1)	2.2 (1.3)	2.3 (0.9)
C_{16}PUFA	0.5^A (0.5)	1.8^B (0.9)	2.4^B (1.2)
C_{18}PUFA	29.5 (7.0)	30.5 (5.1)	31.4 (5.4)
C_{20}PUFA	16.1^A (2.5)	7.1^B (3.9)	10.6^B (2.4)
C_{22}PUFA	9.5^A (3.6)	2.4^B (2.7)	2.8^B (1.7)
sum n-6	4.3 (4.0)	4.6 (2.9)	4.6 (1.8)
sum n-3	50.4^A (8.4)	36.0^B (4.1)	40.6^{AB} (7.3)

† Excluded from the FASTAR analysis. Not present in the reference library.

(9999 permutations) with type III sums of squares. The Spearman correlation coefficient was calculated between the estimates of phytoplankton community composition from FASTAR and microscopy. Seasonal differences in the proportion of specific fatty acids, phytoplankton and bacteria biomass, concentrations of nutrients, chlorophyll *a*, and DOC as well as temperature were tested with ANOVA followed by post hoc tests (Tukey HSD or Games-Howell if the variances were unequal). Multivariate statistical analyses were performed with Primer 6 with PERMANOVA add on (Anderson et al. 2008), and other statistics were performed with SPSS version 21.

Microscopy

Viable phytoplankton and heterotroph cells were counted with the Utermöhl-method of settling a 25–50 mL seston subsample (Utermöhl 1958). All the cells were counted and measured from 25 fields with two magnifications (500× small cells, 250× large cells) with an inverted microscope. The biovolumes were converted to carbon biomass based on the equations from Menden-Deuer and Lessard (2000). The contribution of heterotrophs (protozoan flagellates and ciliates) to total viable cell counts (i.e., including phytoplankton and heterotrophs) was also estimated.

RESULTS

Reference library

The fatty acids from total lipids (TFA) and PLFA of cultured algae had similar taxon-specific fatty acid composition, and grouped according to the taxa, not lipid fractions (Fig. 1). Published fatty acid data (Ahlgren et al. 1992, Taipale et al. 2013) was supplemented with additional original fatty acid analyses from cyanobacteria (*Anabaena, Aphanothece, Microcystis, Planktothrix, Pseudanabaena* and *Synechococcus*) and a dinoflagellate (*Peridinium*). The most abundant fatty acids in cyanobacteria were 16:0, 18:3n-3, 16:1n-7 and 14:0, which accounted for ~72.8% of all fatty acids (Appendix B). In *Peridinium* the most abundant fatty acids were 22:6n-3, 16:0 and 20:5n-3, which accounted for ~64.0% of all fatty acids (Appendix B). The fatty acid profiles of the entire reference library (including also literature data) are available as supplementary material (Appendix C).

The reference library for each algal group should preferably include most of the genera found in the field samples. The similarity of genera between the library and lake samples varied for different phytoplankton groups (Table 1). For cryptophytes and raphidophytes the library contained all the genera that were found
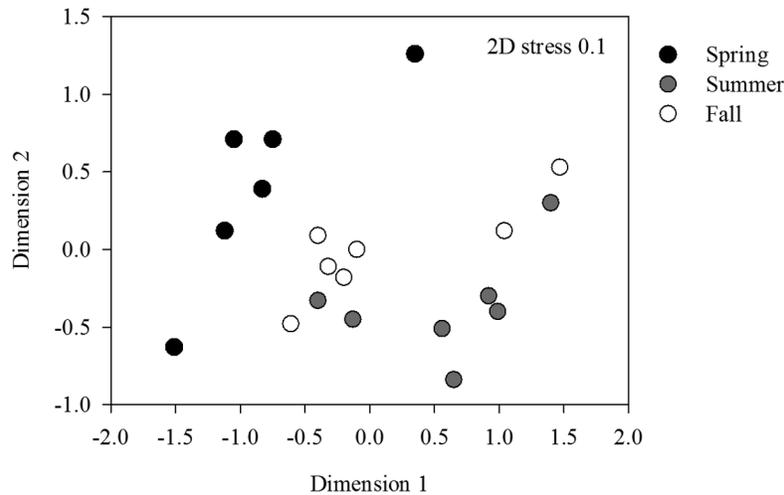
Fig. 1. MDS plot, based on Euclidean distances, of fatty acid composition in total lipids (TFA) and in phospholipids (PLFA) of cultured diatoms, chrysophytes, chlorophytes, dinoflagellates and cryptophytes.

in the lake samples. For chlorophytes only ~13% of the genera in the lake sample were included in the library (Table 1). However, most of these genera were not abundant in the samples and ~39% of the chlorophyte biomass consisted of genera that were included in the library. Similarly, the library contained ~33% of the diatom genera identified with microscopy, but the library contained the most abundant genera (~79% of the diatom biomass consisted of genera that were included the library). Estimation of phytoplankton community composition could be done only for those taxa that were represented in the reference library. Based on the microscopic counts, 0–3% of the phytoplankton biomass was comprised of algae which were not represented in the reference library. The microscopically identified algal classes in the field samples without reference library representation included Prymnesiophyceae, Prasinophyceae, Xantophyceae and Conjugatophyceae, thus the proportion of these classes in the seston could not be modelled with FASTAR. Additionally, unidentified algae constituted ~3% (SD3%) of the phytoplankton in the lake samples.

Phospholipid-derived fatty acids in seston

The most abundant fatty acids in the seston were PUFA, accounting for ~40–55% of all fatty acids (Table 2). The most abundant PUFA were the C_{18} PUFA (18:2n-6, 18:3n-3, 18:4n-3 and 18:5n-3), followed by C_{20} PUFA (mainly 20:4n-6 and 20:5n-3) and C_{22} PUFA (22:5n-6 and 22:6n-3). Saturated fatty acids (SFA) accounted for 25–40% and monounsaturated fatty acids (MUFA) ~15–20% of all fatty acids. The proportion of branched and odd chained fatty acids (typical for heterotrophic bacteria) was only ~2% of all fatty acids. Seston fatty acid composition differed between seasons (PERMANOVA, pseudo-$F_{2,17} = 4.087$, P = 0.001), but not between sampling locations (pseudo-$F_{4,15} = 1.378$, P = 0.189). Specifically the seston fatty acid composition in the spring samples differed from that for summer and fall samples (Fig. 2; Appendix D). The MDS dimension 1 correlated negatively with PUFA, specifically with 20:5n-3 ($r_s = -0.839$, P < 0.001), 22:5n-6 ($r_s = -0.741$, P = 0.001) and 22:6n-3 ($r_s = -0.886$, P < 0.001), and positively with SFA 16:0 ($r_s = 0.878$, P < 0.001) and 18:0 ($r_s = 0.681$, P = 0.001), and MUFA 18:1n-7 ($r_s = 0.583$, P = 0.007) and 18:1n-9 ($r_s = 0.471$, P = 0.036). The seston contained higher proportions of C_{20} PUFA and C_{22} PUFA (specifically 20:5n-3 and 22:6n-3) in the spring than in the summer and fall, while the summer and fall samples contained proportionally more C_{16} PUFA, 16:0 and 18:1n-9 (Table 2).

Phytoplankton community composition

The carbon biomass of phytoplankton was high in the spring and summer and the lowest in the fall (Table 3). The biomass of heterotrophs
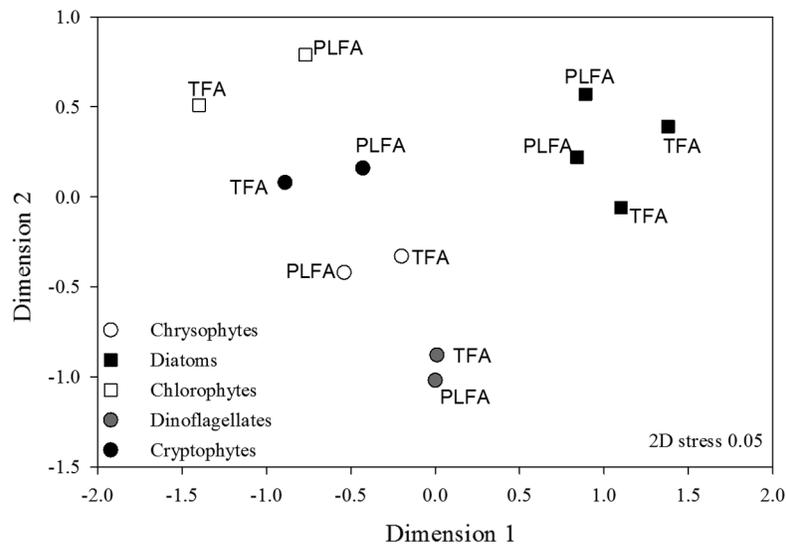
Fig. 2. MDS plot of fatty acid composition (23 PLFA) of seston samples in spring, summer and fall (n = 20).

did not show similar seasonal variation. Phytoplankton constituted ~89–93% of the carbon biomass of viable cells, while ciliates and other heterotrophs accounted for the remaining 7–11%.

Based on microscopic counts and the FASTAR analysis, the major phytoplankton groups were cryptophytes and diatoms, comprising on average 54–63% of the phytoplankton biomass (Fig. 3; Appendix E). According to the microscopic counts, the most dominant diatom genera were *Asterionella*, *Aulacoseira* and *Cyclotella*, and the only identified cryptophyte genera were *Cryptomonas* and *Rhodomonas* (Table 1). Seasonal dynamics of the phytoplankton community composition was observed with both methods. Similar to the seston PLFA, the phytoplankton community composition in spring differed from

that of the summer and fall (Appendix D). Dinoflagellates were more abundant in spring than in the summer and fall, while the proportion of chlorophytes and cyanobacteria increased from spring to summer and fall (Fig. 3). The cyanobacteria were dominated by *Aphanizomenon*, *Anabaena* and *Planktothrix*, while the most abundant chlorophytes were *Chlamydomonas*, *Pediastrum*, *Monoraphidium* and *Oocystis* (Table 1). The three dinoflagellate taxa identified were *Glenodinium*, *Peridinium* and *Gymnodinium*. Estimates for chrysophytes differed between techniques; microscopic counting resulted in significantly higher proportions of chrysophytes than did the FASTAR model (Fig. 3). Chrysophytes were a diverse group; but the most abundant chrysophytes were *Pseudopedinella*

Table 3. Carbon biomass of phytoplankton and heterotrophs, proportion of heterotrophs of living cells in seston in spring, summer and fall (mean with SD in parentheses). Chl *a*, total phosphorus and nitrogen, DOC concentration and surface water temperature are also presented for the different seasons. Different letters indicate significant difference between sampling periods (ANOVA followed by Tukey HSD, P < 0.05).

Characteristic	Spring	Summer	Fall
Phytoplankton (μg C L^{-1})	55.8^A (26.6)	49.9^A (24.8)	22.4^B (7.7)
Heterotrophs (μg C L^{-1})	5.0 (3.5)	3.5 (1.1)	2.7 (0.9)
Percentage of heterotrophs	8.1^{AB} (2.9)	7.0^A (2.9)	11.1^B (3.4)
Chl *a* (μg L^{-1})	1.8 (0.9)	4.1 (2.3)	3.2 (1.8)
Total N (μg L^{-1})	498 (175)	395 (92)	479 (133)
Total P (μg L^{-1})	12.5^A (3.6)	11.4^A (3.4)	5.9^B (3.5)
DOC (mg C L^{-1})	9.2 (1.4)	8.8 (0.9)	8.8 (1.1)
Temperature (°C)	9.8^A (2.4)	20.3^B (0.5)	12.8^C (0.5)
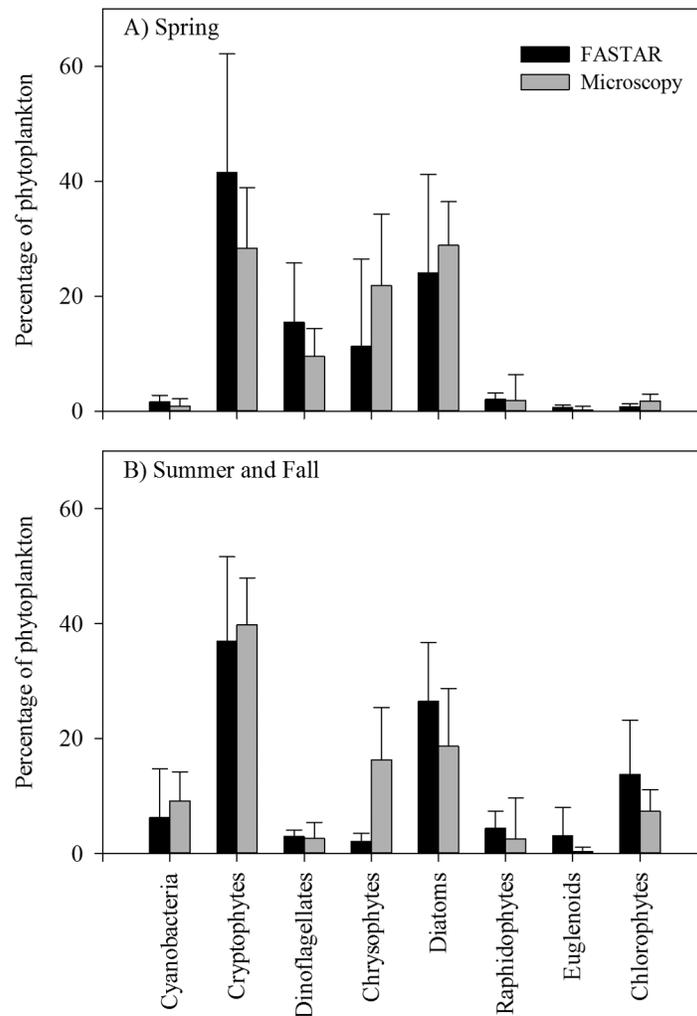
Fig. 3. Average phytoplankton community composition in moderately humic boreal large lakes: (A) spring samples (n = 6) and (B) summer and fall samples (n = 14), error bars represent SD. Summer and fall samples did not differ from each other and were combined. FASTAR results are based on the median model results of percentages of phospholipid-derived fatty acids and the microscopy results are percentages of carbon biomass.

and *Mallomonas*, accounting for ~36% and ~33% of the carbon biomass in chrysophytes, respectively. *Mallomonas* was included in the reference library, but no fatty acid data was available for freshwater *Pseudopedinella*. In general, the community composition estimates for the two techniques were strongly correlated ($r_s = 0.596$, P < 0.001; Fig. 4). Despite the seasonal variation in the community composition, diatoms and cryptophytes were the most abundant algal groups throughout the sampling period.

Discussion

Phytoplankton composition and dynamics respond to environmental changes, such as eutrophication and climate change (Moss et al. 2003, Thackeray et al. 2008). Cyanobacteria are expected to benefit from warmer temperatures and longer growing seasons (Markensten et al. 2010), and eutrophication also shifts phytoplankton community composition towards bloom-forming cyanobacteria (Smith 2003). Cyanobacteria lack 20:5n-3 and 22:6n-3, suggesting that the
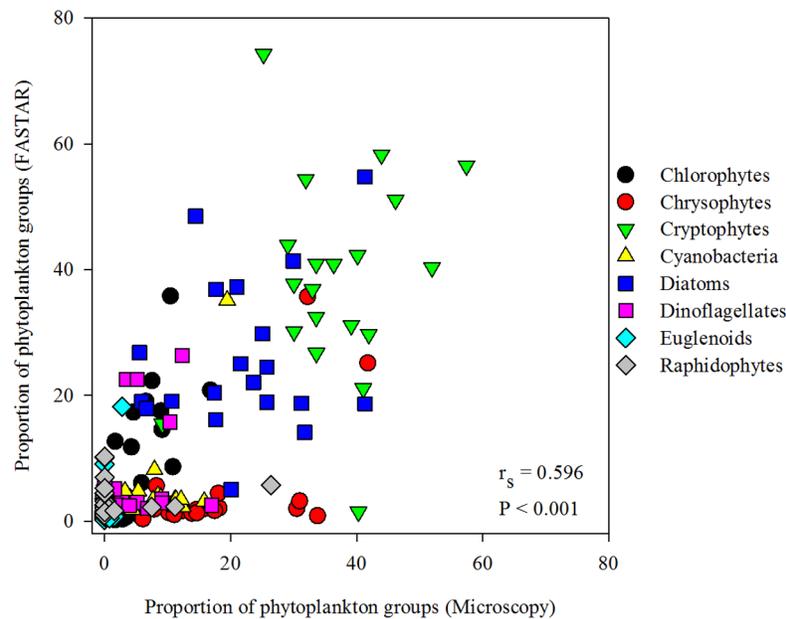
Fig. 4. Correlation of the phytoplankton group proportions from FASTAR modeling (median value) and microscopic counts.

availability of essential fatty acids for consumers may decline as a consequence of climate change and eutrophication of lakes. Application of a biochemical technique coupled with modeling makes it possible to study both compositional changes in the phytoplankton as well as the changes in the nutritional quality of the seston. Microscopic counts and FASTAR modeling gave similar estimates for the phytoplankton community composition in the studied lakes (Figs. 3 and 4). This result indicates that the taxon-specificity of algae is not only a trait of cultured algae, but persists also in nature, and that phospholipid-derived fatty acids can be used as chemotaxonomic markers within a Bayesian mixing model framework. Single fatty acid biomarkers are not always diagnostic for specific phytoplankton groups, as only few fatty acids are truly taxon-specific (Sushchik et al. 2004, Taipale et al. 2013, Bi et al. 2014). Multivariate approaches utilize the entire fatty acid composition, and thus are more robust than the single fatty acid biomarker methods.

Both the mixing model and microscopy methods indicated that the proportion of dinoflagellates peaked in the spring, while the abundance of chlorophytes and cyanobacteria increased from the spring to the summer and fall (Fig. 3). Seasonal changes in seston fatty acid composition coincided with the phytoplankton dynamics. Even when the changes were relatively small, the FASTAR analysis was sensitive enough to detect the seasonal variation in the seston fatty acid composition and accordingly predict changes in the phytoplankton community composition. The increased proportion of chlorophytes and cyanobacteria in the summer and fall corresponds with lower proportions of PUFA, i.e., the seston quality as a food source for zooplankton declined (Müller-Navarra et al. 2000).

The high proportions of C_{20} and C_{22} PUFA in the spring were related to the abundance of diatoms and flagellates. The abundance of docosahexaenoic acid (22:6n-3, DHA) in spring samples corresponds with the prevalence of dinoflagellates, as indicated by both the mixing model and microscopic counts. Dinoflagellates are rich in DHA, and a similar connection between the seston DHA concentration and abundance of dinoflagellates have been previously noted (Ahlgren et al. 1997, Gutseit et al. 2007). Also the proportion of EPA in the seston was the highest in the spring. A high proportion of EPA is typically associated with diatoms,

although other algae also contain EPA, e.g., in cryptophytes and dinoflagellates EPA accounts for ~10% of total fatty acids (Ahlgren et al. 1992, Taipale et a. 2013). The multivariate approach used in the current study indicates that the changes in the seston EPA are also due to variation in the abundance of dinoflagellates. The proportion of C_{20} and C_{22} PUFA decreased towards the summer and fall, whereas the proportions of SFA, MUFA and C_{16} and C_{18} PUFA increased. FASTAR indicated that these changes corresponded with decreasing proportions of dinoflagellates and increasing proportions of chlorophytes and cyanobacteria.

The mixing model approach significantly underestimated the proportions of chrysophytes compared to the results from the traditional microscopic method. The discrepancy between methods for the estimation of chrysophytes may be due to compositional similarity of chrysophytes and cryptophytes (Taipale et al. 2013), an incomplete reference library or the presence of mixotrophy (Isaksson 1998). These differences could also be due to error in the microscopic counts as the Utermöhl method is very time intensive so only a very small sample volume is actually enumerated. Both chrysophytes and cryptophytes are rich in PUFA (specifically the C_{18} PUFA 18:4n-3 and 18:3n-3, but also several C_{20} and C_{22} PUFA), making it difficult to separate these algal groups using fatty acids (Taipale et al. 2013). An appropriate reference library is essential for mixing model analyses of biomarkers; FASTAR will always assign the fatty acids to one of the algal groups in the reference library and thus the library should contain the phytoplankton groups that are most abundant in the lake samples. To include as much within class variation as possible, several genera from the same group should be included in the library. In the current study the reference library is presumed to be adequate for diatoms, cryptophytes, chlorophytes, raphidophytes, dinoflagellates and cyanobacteria, since the library included fatty acid data on the most abundant taxa identified in the field samples (Table 1). Species specific variation within the group is accounted for in the above mention phytoplankton groups. However, the reference library for chrysophytes is incomplete as the fatty acid profile for the most abundant chrysophyte, *Pseudopedinella* (compris-

ing ~36% of chrysophyte biomass in the field samples), is missing and literature values for this taxa were not found for freshwater strains. Fatty acid data on marine *Pseudopedinella* differed from that of the reference chrysophyte (Chuecas and Riley 1969), specifically the proportions of C_{22} PUFA were significantly lower in the marine *Pseudopedinella* than in chrysophytes in the reference library (Taipale et al. 2013). Algal fatty acid composition is strongly influenced by phylogeny, suggesting that the proportion of DHA and 22:5n-6 might be low also in freshwater *Pseudopedinella*. The presence of mixotrophy may also confound the FASTAR result for chrysophytes. The conditions of algal cultures were predominantly autotrophic, but the cultures were not axenic and a small degree of mixotrophy of e.g., *Dinobryon* may be accounted in the reference library (Ahlgren et al. 1992, Taipale et al. 2013). Effects of mixotrophy on the growth and production of chrysophytes is highly taxon-specific. The second most abundant chrysophyte in the lake samples was *Mallomonas* (~33% of the carbon biomass in chrysophytes), which is an obligate photoautotroph, thus excluding mixotrophy as a possible confounding factor for this genus (Rottberger et al. 2013).

The reference library consisted of total fatty acids of cultured algae. The TFA and PLFA fractions of cultured algae had similar taxon-specific fatty acid composition, and grouped according to the taxa, not lipid fractions (Fig. 1). Thus, previously reported TFA profiles from monocultures of chrysophytes, diatoms, chlorophytes, euglenoids, cryptophytes, raphidophytes, and dinoflagellates could be used to build the reference library for FASTAR analyses (Ahlgren et al. 1992, Taipale et al. 2013). The similarity between TFA and PLFA of algal cultures was previously noted by Dijkman and Kromkamp (2006).

Seston is highly variable, and in addition to phytoplankton, seston in both freshwater and marine habitats contains detritus and heterotrophs, which also contribute to the fatty acid composition (Bec et al. 2010*a*, 2010*b*, Lowe et al. 2014). In humic lakes, detritus is known to comprise of a wide variety of molecular components, including also free fatty acids (Schulten and Gleixner 1999). The detrital fatty acids may account for up to 6% of total fatty acids in seston

(Bec et al. 2010b). The solid-phase extraction of total lipids to achieve PLFA effectively excludes detrital fatty acids; as indicated by the absence of long chain saturated fatty acids ($>C_{20}$) and the trace proportions of C_{20} SFA (0.1% of all fatty acids) that have been suggested to originate from terrestrial detritus (Brett et al. 2009). However, PLFA analysis cannot differentiate photosynthetic algae from planktonic heterotrophs. The microbial food web (i.e., trophic pathway that transfers DOC via bacteria to upper trophic levels) has been suggested to be significant in lakes with high DOC concentrations (Berggren et al. 2010). PLFA from non-algal sources may confound the use of PLFA as chemotaxonomic marker for phytoplankton. The proportion of exclusively bacterial fatty acids (branched and odd-chained fatty acids) in the lake seston was low (1–2% of all fatty acids), suggesting that fatty acids from heterotrophic bacteria will not interfere with the estimates of phytoplankton composition even with moderate DOC concentrations ($\sim$9 mg C L^{-1}). The potentially confounding effect of heterotrophs to the seston PLFA composition is currently not possible to estimate with this modeling approach due to the lack of an appropriate reference library. Microscopic counting estimated that heterotrophs accounted for 7–11% of the carbon biomass of all viable cells. The similarity between the estimations from the fatty acid based mixing model and microscopic counting suggests that the contribution of PLFA from heterotrophs is low or that the PLFA from heterotrophs are similar to those from phytoplankton. Laboratory experiments indicate the fatty acid composition of heterotrophs is strongly influenced by the diet, although de novo synthesis of C_{20} and C_{22} PUFA may occur if the diet is deprived of these fatty acids (Bec et al. 2010a). However, it is unclear how prevalent this metabolic pathway is when long chain PUFA are readily available in the diet (Bec et al. 2010a).

Phytoplankton which are not represented in the reference library were excluded from the model estimations (i.e., Prasinophyceae, Xantophyceae, Prymnesiophyceae, and Conjugatophyceae), but these accounted for only 0–3% of the phytoplankton community composition. The absence of small taxonomic groups from the model analysis does not seem to have a major effect on the estimates of the phytoplankton community composition (Fig. 3). Large credibility intervals or multi-modal posterior density distributions may indicate uncertainty in the FASTAR estimates of the phytoplankton community composition (Appendix E). Despite the methodological differences between the two techniques, the mixing model approach and microscopy provided similar estimates for the phytoplankton community composition. A larger volume of water (1L or more if needed) is sampled thoroughly in a biochemical extraction procedure relative to the microscopy approach. Both techniques require substantial training, but the mixing model resource library can be adapted to different ecosystems by adjusting the reference library, and also the comparison of results from different studies can be improved by standardization of the biochemical analyses and mixing model parameters. Moreover, the mixing model approach should be largely exempt from between-analyst biases that will always, to varying degrees, occur with microscopic counts.

Fatty acids and phytoplankton in moderately humic lakes: composition and dynamics

The phytoplankton community composition in the studied lakes was dominated by flagellates and diatoms. In large lakes, such as the study lakes, extensive turbulence favors diatoms (Lepistö and Rosenström 1998). Reduced light conditions may favor species that are able adjust their position in the water column and also use alternative energy sources, hence humic lakes are typically dominated by flagellates, especially cryptophytes and chrysophytes. In comparison to photoautotrophy, direct bacterivory seems to be insignificant in the energy balance or carbon budget of mixotrophs, but may serve as source of phosphorus and nitrogen when the supply of inorganic nutrients is limited (Tranvik et al. 1989, Nygaard and Tobiesen 1993).

Applying fatty acids to estimate phytoplankton community composition also provides information on the biochemical composition of the seston. The nutritional quality of algae is related to its elemental and biochemical composition, e.g., the fatty acid composition, particularly the proportion of PUFA from the n-3 and n-6 families. Algae are the major producers of n-3 and n-6 PUFA and seston rich in PUFA have been shown to be a high quality resource for zoo-

plankton (Müller-Navarra et al. 2000). The results of the current study indicate that in terms of fatty acid composition, the quality of potential zooplankton food varies and the highest quality food in these lakes occurred in the spring, when the proportion of C_{20} and C_{22} PUFA peaked (Table 2). The seasonal changes in the seston PUFA were most pronounced for DHA, which peaked at ~8% in the spring and declined to ~2% in the summer and fall (Table 2). The importance of algae as a food source to zooplankton is not determined only by its nutritional quality, but also the size and morphology (Burns 1968). The modeling results indicate that the increased availability of DHA in the spring is due to the abundance of dinoflagellates. Freshwater dinoflagellates are not considered to be toxic to zooplankton and are within the edible size range for larger zooplankton (Rengefors and Legrand 2001). However, for smaller zooplankton dinoflagellates may be too large to ingest (Rengefors and Legrand 2001). Complementing seston fatty acid composition with information on the phytoplankton community composition improves the evaluation of the availability of PUFA to micro- and macrozooplankton. The total availability of PUFA to upper trophic level consumers decreased from the spring to the summer and fall, as the phytoplankton composition changed. Similar seasonal dynamics of PUFA have also been documented in other freshwater and coastal marine phytoplankton communities (Ahlgren et al. 1997, Lowe et al. 2014). In the summer the phytoplankton biomass was similar to that of spring samples, but the quality declined, because the proportion of DHA and EPA decreased. Changes in the seston fatty acid composition and the low phytoplankton biomass in the fall indicate seasonal variation in the availability of EPA and DHA for consumers. Note that despite the declining trend of PUFA availability, due to the abundance of diatoms and cryptophytes, EPA and DHA were readily available in the food web of large lakes, accounting for about 8–10% of PLFA also in the summer and fall. The seasonal variation in the quantity and quality of seston indicates the importance of the biochemical composition of seston for the understanding of the composition and dynamics of pelagic food webs in lakes.

LITERATURE CITED

Ahlgren, G., W. Goedkoop, H. Markensten, L. Sonesten, and M. Boberg. 1997. Seasonal variation in food quality for pelagic and benthic invertebrates in Lake Erken: the role of fatty acids. Freshwater Biology 38:555–570.

Ahlgren, G., I.-B. Gustafsson, and M. Boberg. 1992. Fatty acid content and chemical composition of freshwater microalgae. Journal of Phycology 28:37–50.

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E Ltd, Plymouth, UK.

Bec, A., D. Martin-Creuzburg, and E. Von Elert. 2010*a*. Fatty acid composition of the heterotrophic nanoflagellate Paraphysomonas sp.:influence of diet and de novo biosynthesis. Aquatic Biology 9:107–112.

Bec, A., M.-E. Perga, C. Desvilettes, and G. Bourdier. 2010*b*. How well can the fatty acid content of the lake seston be predicted from its taxonomic composition? Freshwater Biology 55:1958–1972.

Berggren, M., L. Ström, H. Laudon, J. Karlsson, A. Jonsson, R. Giesler, A.-K. Bergström, and M. Jansson. 2010. Lake secondary production fueled by rapid trasnfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. Ecology Letters 13:870–880.

Brett, M. T., M. J. Kainz, S. J. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proceedings of the National Academy of Sciences USA 106:21197–21201.

Bi, R., C. Arnd, and U. Sommer. 2014. Linking elements to biochemical: effects of nutrient supply ratios and growth rates on fatty acid composition of phytoplankton species. Journal of Phycology 50:117–130.

Birk, S., W. Bonne, A. Borja, S. Brucet, A. Courrat, S. Poikane, A. Solimini, W. van de Bund, N. Zampoukas, and D. Hering. 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. Ecological Indicators 18:21–41.

Boschker, H. T. S., J. C. Kromkamp, and J. J. Middelburg. 2005. Biomarker and carbon isotopic con-

straints on bacterial and algal community structure and functioning in a turbid, tidal estuary. Limnology and Oceanography 50:70–80.

Burns, C. W. 1968. The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. Limnology and Oceanography 13:675–678.

Chuecas, L., and J. P. Riley. 1969. Component fatty acids of the total lipids of some marine phytoplankton. Journal of the Marine Biological Association of the United Kingdom 49:97–116.

Dalsgaard, J., M. St John, G. Kattner, D. Müller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46:225–340.

Dijkman, N. A., and J. C. Kromkamp. 2006. Phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton: application for inferring phytoplankton composition. Marine Ecology Progress Series 324:113–125.

Frostegård, Å., A. Tunlid, and E. Bååth. 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43:1621–1625.

Galloway, A. W. E., M. E. Eisenlord, M. N. Dethier, G. Holtgrieve, and M. T. Brett. 2014a. Quantitative estimates of resource utilization by an herbivorous isopod using a Bayesian fatty acid mixing model. Marine Ecology Progress Series 507:219–232.

Galloway, A. W. E., S. J. Taipale, M. Hiltunen, E. Peltomaa, U. Strandberg, M. T. Brett, and P. Kankaala. 2014b. Diet specific biomarkers show that high quality phytoplankton fuel herbivorous zooplankton in large boreal lakes. Freshwater Biology 59:1902–1915.

Gregor, J., and B. Maršálek. 2004. Freshwater phytoplankton quantification by chlorophyll a: a comparative study of in vitro, in vivo and in situ methods. Water Research 38:517–522.

Gutseit, K., O. Berglund, and W. Granéli. 2007. Essential fatty acids and phosphorus in seston from lakes with contrasting terrestrial dissolved organic carbon content. Freshwater Biology 52:28–38.

Isaksson, A. 1998. Phagotrophic phytoflagellates in lakes: a literature review. Archiv für Hydrobiologie Special Issues: Advances in Limnology 51:63–90.

Lepistö, L., and U. Rosenström. 1998. The most typical phytoplankton taxa in four types of boreal lakes. Hydrobiologia 369/370:89–97.

Léveillé, J.-C., C. Amblard, and G. Bourdier. 1997. Fatty acid as specific algal markers in a natural lacustrian phytoplankton. Journal of Plankton Research 19:469–490.

Lowe, A. T., A. W. E. Galloway, J. S. Yeung, M. N. Dethier, and D. O. Duggins. 2014. Broad sampling and diverse biomarkers allow characterization of nearshore particulate organic matter. Oikos 123:1341–1354.

MacIntyre, H. L., E. Lawrenz, and T. L. Richardson. 2010. Taxonomic discrimination of phytoplankton by spectral fluorescence. Pages 129–169 in D. J. Suggett, O. Prasil, and M. A. Borowitzka, editors. Chlorophyll fluorescence in aquatic sciences: Methods and applications. Springer, Berlin, Germany.

Mackey, M. D., D. J. Mackey, H. W. Higgins, and S. W. Wright. 1996. CHEMTAX: a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Marine Ecology Progress Series 144:265–283.

Markensten, H., K. Moore, and I. Persson. 2010. Simulated lake phytoplankton composition shifts towards cyanobacteria dominance in a future warmer climate. Ecological Applications 20:752–767.

McCormick, P. V., and J. Cairns. 1994. Algae as indicators of environmental change. Journal of Applied Phycology 6:509–526.

Menden-Deuer, S., and E. J. Lessard. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protest plankton. Limnology and Oceanography 45:569–579.

Moore, J. W., and B. X. Semmens. 2008. Incorporation uncertainty and prior information into stable isotope mixing models. Ecology Letters 11:470–480.

Moss, B., D. Mckee, D. Atkinson, S. .E. Collings, J. W. Eaton, A. B. Gill, I. Harvey, K. Hatton, T. Heyes, and D. Wilson. 2003. How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms. Journal of Applied Ecology 40:782–792.

Müller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74–77.

Nygaard, K., and A. Tobiesen. 1993. Bacterivory in algae: a survival strategy during nutrient limitation. Limnology and Oceanography 38:273–279.

Piepho, M., M. T. Arts, and A. Wacker. 2012. Species-specific variation in fatty acid concentrations of four phytoplankton species: Does phosphorus supply influence the effect of light intensity or temperature? Journal of Phycology 48:64–73.

R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reitan, K. I., J. R. Rainuzzo, and Y. Olsen. 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. Journal of Phycology 30:972–979.

Rengefors, K., and C. Legrand. 2001. Toxicity in Peridinium aciculiferum: an adaptive strategy to outcompete other winter phytoplankton. Limnology and Oceanography 46:1990–1997.

Reuss, N., and L. K. Poulsen. 2002. Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. Marine Biology 141:423–434.

Reynolds, C. S., V. Huszar, C. Kruk, L. Naselli-Flores, and S. Melo. 2002. Towards a functional classification of the freshwater phytoplankton. Journal of Plankton Research 24:417–428.

Rottberger, J., A. Gruber, J. Boenigk, and P. G. Kroth. 2013. Influence of nutrients and light on autotrophic, mixotrophic and heterotrophic freshwater chrysophytes. Aquatic Microbial Ecology 71:179–191.

Schlüter, L., T. L. Lauridsen, G. Krogh, and T. Jørgensen. 2006. Identification and quantification of phytoplankton groups in lakes using new pigment ratios: a comparison between pigment analysis by HPLC and microscopy. Freshwater Biology 51:1474–1485.

Schulten, H.-R., and G. Gleixner. 1999. Analytical pyrolysis of humic substances and dissolved organic matter in aquatic systems: structure and origin. Water Research 33:2489–2498.

Smith, V. N. 2003. Eutrophication of freshwater and coastal marine ecosystems: A global problem. Environmental Science and Pollution. 10:126–139.

Steinberg, M. K., M. R. First, E. J. Lemieux, L. A. Drake, B. N. Nelson, D. M. Kulis, D. M. Anderson, and N. A. Welschmeyer. 2012. Comparison of techniques used to count single-celled viable phytoplankton. Journal of Applied Phycology 24:751–758.

Sushchik, N. N., M. I. Gladyshev, O. N. Makhutova, G. S. Kalachova, E. S. Kravchuk, and E. A. Ivanova.

2004. Associating particulate essential fatty acids of the ω3 family with phytoplankton species composition in a Siberian reservoir. Freshwater Biology 49:1206–1219.

Taipale, S., U. Strandberg, E. Peltomaa, A. W. E. Galloway, A. Ojala, and M. T. Brett. 2013. Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquatic Microbial Ecology 71:165–178.

Thackeray, S. J., I. D. Jones, and S. C. Maberly. 2008. Long-term change in the phenology of spring phytoplankton: species-specific responses to nutrient enrichment and climate change. Journal of Ecology 96:523–535.

Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11:107–184.

Tranvik, L. J., K. G. Porter, and J. McN. Sieburth. 1989. Occurrence of bacterivory in Cryptomonas, a common freshwater phytoplankter. Oecologia 78:473–476.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen phytoplankton: methodik. Mitteilungen der internationale Vereinigung für theoretische und angewandte Limnologie 9:1–38.

Van den Meersche, K., K. Soetaert, and J. J. Middelburg. 2008. A Bayesian compositional estimator for microbial taxonomy based on biomarkers. Limnology and Oceanography: Methods 6:190–199.

Zelles, L. 1999. Fatty acid patterns of phospholipid and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology and Fertility of Soils 29:111–129.

SUPPLEMENTAL MATERIAL

APPENDIX A

Table A1. Location as coordinates, mean depth and total lake area of the studied lakes as well as mean (SD) of Secchi depth, dissolved organic carbon (DOC), total nitrogen (total N), total phosphorus (total P), and chlorophyll a (Chl a) of the five sampled lake basins in the eastern Finland. Each lake basin had one sampling location except Orivesi and Suvasvesi, which had two sampling locations. The water parameter values are pooled for 0–4 m depth sampled in end of May, early August and end of September 2011.

Lake	Location	Mean depth (m)	Area (km^2)	Secchi depth (m)	DOC (mg L^{-1})	Total N (µg L^{-1})	Total P (µg L^{-1})	Chl a (µg L$^-$)
Orivesi	62°10′ N; 29°43′ E	9	601	2.1 (0.3)	7.0 (0.3)	304 (32)	11.8 (1.0)	6.3 (2.6)
Paasivesi	62° 8′ N; 29°26′ E	21	124	2.5 (0.5)	8.4 (0.4)	349 (27)	5.7 (3.2)	2.0 (1.1)
Suvasvesi	62°40′ N; 28°12′ E	10.4	233	3.1 (0.5)	8.8 (0.4)	490 (42)	10.0 (7.3)	2.8 (1.0)
Pyhäselkä	62°28′ N; 29°48′ E	8.8	361	1.9 (0.0)	9.5 (0.5)	399 (48)	9.3 (0.6)	1.5 (0.8)
Kallavesi	62°49′ N; 27°52′ E	8.6	478	2.3 (0.6)	10.2 (0.2)	639 (107)	12.1 (7.1)	3.4 (1.3)

APPENDIX B

Table B1. Fatty acid profiles of algal monocultures of 6 cyanobacteria strains and a dinoflagellate. Values are percentages of total fatty acids.

	Cyanobacteria						Dinoflagellate
Fatty acid	*Microcystis*	*Pseudanabaena*	*Anabaena*	*Aphanothece*	*Planktothrix*	*Pseudanabaena* SSCAP K-1230	*Peridinium*
14:0	0.1	30.7	10.4	13.9	0.2	22.6	7.4
16:0	46.7	18.2	36.3	24.7	33.8	12.8	24.1
16:1n-9	0.6	1.0	1.5	0	0.5	0	0.1
16:1n-7	1.9	7.2	4.0	40.7	23.5	3.0	2.1
17:0	0	0	0	0.8	0.2	0.2	0
16:3n-3	0	0	6.9	0	0	0	0
16:4n-3	0	0.1	0	0	0	0	0.4
18:0	1.3	1.6	2.1	1.9	3.7	4.0	1.2
18:1n-9	1.8	0.6	0.8	0.2	1.0	2.6	6.4
18:1n-7	0.1	13.0	2.0	7.7	4.0	20.2	1.7
18:2n-6	4.5	1.1	2.7	0	3.2	1.3	0.2
18:3n-6	21.4	1.1	0.5	0	0	0	0.1
18:3n-3	6.7	18.6	28.5	0	27.0	25.0	0.6
18:4n-3	12.8	0	0	0	0	0	10.6
18:5n-3	0	0	0	0	0	0	4.0
20:0	0	0	0	0.1	0.1	0.1	0
20:4n-3	1.1	0	0	0	0	0	0
20:5n-3	0	0	0	0	0	0	17.7
22:6n-3	0	0	0	0	0	0	22.2
SFA	48.2	50.5	48.8	41.4	38.0	39.6	32.6
MUFA	4.5	21.8	8.2	48.6	29.1	25.7	10.3
PUFA	46.4	20.8	38.6	0.0	30.2	26.3	55.8
Bacterial FA	0.0	0.0	0.0	0.8	0.2	0.2	0.0
C$_{16}$PUFA	0.0	0.1	6.9	0.0	0.0	0.0	0.4
C$_{18}$PUFA	45.3	20.7	31.8	0.0	30.2	26.3	15.5
C$_{20}$PUFA	1.1	0.0	0.0	0.0	0.0	0.0	17.7
C$_{22}$PUFA	0.0	0.0	0.0	0.0	0.0	0.0	22.2
sum n-6	25.8	2.1	3.3	0.0	3.2	1.3	0.3
sum n-3	20.6	18.7	35.4	0.0	27.0	25.0	55.5

Appendix C

Table C1. Reference library that was used to infer the phytoplankton composition in the field sample. Data was obtained from the current study (cyanobacteria n = 6, dinoflagellates n = 1), and from published literature values: Taipale et al. 2013 (cryptophytes n = 9, chrysophytes n = 3, diatoms n = 7, raphidophytes n = 1, euglenoids n = 3, chlorophytes n = 11) and from Ahlgren et al. 1992 (dinoflagellates n = 2). Values are percentages of total fatty acids (mean with SD in parentheses).

Fatty acid	Cyanobacteria	Cryptophytes	Dinoflagellates	Chrysophytes	Diatoms	Raphidophyte†	Euglenoids	Chlorophytes
14:0	13.0(12.2)	3.7(5.2)	9.0(3.0)	22.3(16.3)	9.2(3.6)	8.1(0.8)	4.3(1.7)	0.7(1.2)
15:0	0.0(0.0)	0.0(0.0)	0.8(0.7)	0.0(0.0)	0.6(0.5)	1.0(0.1)	2.3(2.6)	0.2(0.4)
16:0	28.7(12.6)	20.9(10.4)	36.4(14.1)	10.3(2.4)	16.8(2.8)	18.5(1.8)	11.8(2.3)	20.0(5.2)
16:1n-9	0.6(0.6)	0.2(0.5)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.1(0.2)
16:1n-7	13.4(15.6)	2.8(3.0)	1.5(0.7)	4.4(6.2)	33.3(5.9)	1.2(0.1)	1.8(0.5)	1.5(2.1)
16:1n-5	0.0(0.0)	0.2(0.7)	0.0(0.0)	0.2(0.2)	0.4(0.4)	1.0(0.1)	0.0(0.0)	1.7(2.9)
16:2n-4	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	2.7(1.7)	7.8(0.8)	0.0(0.0)	0.0(0.0)
16:3n-4	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	6.6(2.1)	1.5(0.1)	0.0(0.0)	0.0(0.0)
16:3n-3	1.1(2.8)	0.1(0.1)	0.0(0.0)	0.2(0.4)	0.0(0.0)	0.0(0.0)	3.3(1.3)	2.4(1.6)
17:0	0.2(0.3)	0.5(0.6)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	1.1(1.2)	0.5(1.3)
16:4n-3	0.0(0.0)	0.1(0.1)	0.1(0.2)	0.0(0.0)	0.0(0.0)	0.0(0.0)	6.3(3.5)	8.9(3.9)
18:0	2.4(1.1)	2.6(1.2)	1.8(1.2)	3.9(3.9)	5.0(5.8)	0.9(0.1)	1.8(0.7)	2.8(2.9)
18:1n-9	1.2(0.9)	2.4(1.3)	19.2(10.5)	3.2(2.5)	1.5(0.8)	0.6(0.1)	2.7(0.5)	13.8(13.7)
18:1n-7	7.8(7.6)	3.1(2.2)	0.8(0.9)	4.3(3.9)	2.1(1.4)	0.2(0.0)	0.6(0.3)	2.8(2.9)
18:2n-6	2.1(1.6)	4.2(3.8)	0.7(0.5)	8.2(1.5)	1.6(1.8)	0.9(0.1)	6.2(5.9)	6.9(4.7)
18:3n-6	3.8(8.6)	0.0(0.0)	0.1(0.1)	0.5(0.7)	0.2(0.3)	0.0(0.0)	0.0(0.0)	0.8(1.9)
18:3n-3	17.6(11.8)	23.7(6.6)	0.2(0.3)	12.0(4.0)	0.1(0.2)	10.0(1.0)	11.4(1.7)	27.6(12.4)
18:4n-3	2.1(5.2)	17.3(10.6)	5.7(3.8)	10.7(6.1)	0.5(1.0)	10.1(1.0)	0.0(0.0)	5.0(3.6)
20:4n-6	0.0(0.0)	0.0(0.0)	0.1(0.1)	1.5(2.5)	1.8(3.0)	3.5(0.3)	5.1(4.1)	0.0(0.0)
20:4n-3	0.2(0.5)	0.2(0.5)	0.0(0.0)	0.7(0.8)	0.0(0.0)	0.0(0.0)	1.8(0.6)	0.0(0.0)
20:5n-3	0.0(0.0)	9.8(4.2)	9.6(7.1)	1.8(3.1)	13.2(5.8)	17.6(1.8)	10.3(2.6)	0.0(0.0)
22:5n-6	0.0(0.0)	3.6(1.6)	0.0(0.0)	8.2(3.4)	0.1(0.2)	0.0(0.0)	3.7(1.3)	0.0(0.0)
22:6n-3	0.0(0.0)	2.8(2.5)	12.8(9.2)	4.5(3.8)	1.2(0.7)	1.1(0.1)	8.7(3.0)	0.0(0.0)

† SD represents within taxa variation.

Appendix D

Table D1. PERMANOVA results of the post-hoc pairwise tests of seston PLFA composition between seasons. Analysis assumes the factor Season is fixed, and uses unrestricted permutation of raw data and type III sums of squares. Significance determined with permutation and Monte Carlo (P(MC)) P-values.

Season	t	P(perm)	Unique perms	P(MC)
Spring, Summer	2.6482	0.0022	1705	0.0009
Spring, Fall	1.8719	0.0051	1710	0.0218
Summer, Fall	1.325	0.1663	1705	0.1638

Table D2. PERMANOVA results of the post-hoc pairwise tests of seston phytoplankton composition (based on microscopic counts) between seasons. Analysis assumes the factor Season is fixed, and uses unrestricted permutation of raw data and type III sums of squares. Significance determined with permutation and Monte Carlo (P(MC)) P-values.

Season	t	P(perm)	Unique perms	P(MC)
Spring, Summer	2.285	0.0008	1711	0.004
Spring, Fall	1.8373	0.0189	1251	0.045
Summer, Fall	1.4588	0.0916	1709	0.0973

APPENDIX E
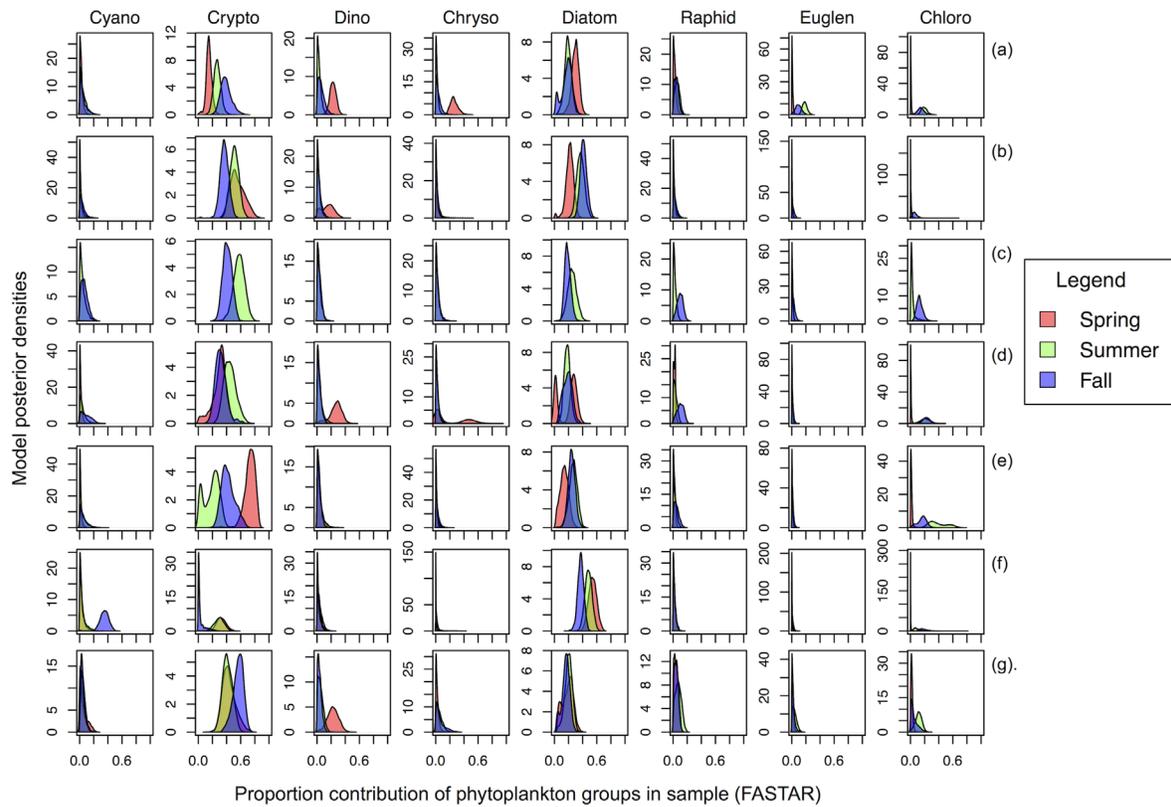
Fig. E1. Posterior distributions from each FASTAR analysis. Locations are (a) Orivesi 107, (b) Orivesi 2, (c) Paasivesi (no spring sample), (d) Suvasvesi 29, (e) Suvasvesi 399, (f) Pyhäselkä, (g) Kallavesi.