Spatial microheterogeneity and selective microbial consumption of dissolved free amino acids in an oligomesotrophic lake

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Abstract

We studied the cm to m scale spatial distribution of dissolved free amino acids (DFAA) in the upper epilimnion of oligomesotrophic Lake Zurich in 14 sampling campaigns over > 3 years and at various periods of the growing season. During each campaign, 10 sets of 10 simultaneously drawn samples (10 mL, 2 cm distance) were collected from 5 m depth. DFAA concentrations varied by one to > 3 orders of magnitude within sets, providing field evidence for DFAA release from macroscopic point sources and for substantial variability of the in-situ growth conditions of bacterioplankton metacommunities. There was a tight relationship between the median DFAA concentration per sampling campaign and the compositional heterogeneity of the 15 most common AA: their composition was similar in samples from campaigns with high median DFAA concentrations, indicating that spatial distribution patterns were mainly a result of physical mixing. By contrast, AA composition was spatially variable in campaigns with low median DFAA concentrations, and serine, aspartate, and glycine were disproportionally high in the 10% samples with highest DFAA concentrations. We hypothesized that pelagic bacteria would preferably target pulses of such locally overrepresented AA. Short-term incubations with radiolabeled tracers revealed substantially higher microbial uptake of serine and, to a lesser extent, aspartate, than of two amino acids with consistently low in situ concentrations (leucine, isoleucine). This illustrates a “preparedness” of the bacterioplankton to preferably incorporate those AAs that are more available in DFAA hotspots.

Dissolved organic carbon represents one of the largest exchangeable carbon reservoirs in aquatic systems (Carlson 2002). It overlaps with the pool of dissolved organic nitrogen in the subset of labile low-molecular-weight compounds that are readily available to microorganisms, such as dissolved free amino acids (DFAAs) (Crawford et al. 1974; Rosenstock and Simon 1993; Kirchman 2003). DFAA concentrations in lake water vary with season, depth, and even time of the day (Jørgensen et al. 1983; Simon 1998; Rosenstock and Simon 2001). Their seasonal variation often reflects that of primary productivity, suggesting that a substantial fraction originates from algal excretion (Brown 1991; Sarmento et al. 2013), zooplankton “sloppy feeding” (Riemann et al. 1986), and the decay of flocculating algal biomass (Grossart and Simon 1993).

Heterotrophic bacteria are the most important DFAA consumers in freshwater, estuarine and marine pelagic ecosystems (Rosenstock and Simon 1993; Kirchman 2003). DFAA uptake significantly contributes to total bacterial production (Bertilsson et al. 2007). It can satisfy the larger part of bacterial biosynthesis and energy demands, and might be even more important for covering microbial N requirements (Keil and Kirchman 1991). Pelagic bacterial communities exploit DFAAs across a large range of concentrations. They may be physiologically “prepared” for an uneven substrate field (Egli 2010) and respond to changing substrate conditions by upregulating the synthesis and activity of enzymes and transporters (Gottschalk 1986; Unanue et al. 1999). Moreover, sympatric populations may differ in their affinities to low DFAA concentrations or uptake capacities for DFAA pulses (Alonso and Pernthaler 2006; Salcher et al. 2011).

Conceptually, DFAAs may originate (more or less) “diffusely” in the pelagic zone, e.g., from single microbial cells (Kawasaki and Benner 2006; Sarmento et al. 2013), from the hydrolysis of proteins, peptides or other forms of dissolved combined amino acids (DCAAs) (Rosenstock and Simon 1993; Simon 1998; Rosenstock and Simon 2001), but also from comparably rare macroscopic point sources (Kiørboe and Jackson 2001; Taylor and Stocker 2012), such as algal-derived organic aggregates (Grossart and Simon 1998; Grossart and Ploug 2001). In the wave-affected surface boundary layer of lakes the subsequent distribution of DFAAs from such discrete
sources across spatial scales of cm to m would be mostly mediated by turbulent mixing processes (Wüest and Lorke 2003). However, data from aggregates collected in situ both support (Grossart and Simon 1998; Aldredge 2000) and refute (Karner and Herndl 1992) the notion that the matrix of organic aggregates is indeed enriched in organic solutes. In fact, it has never been shown that there is measurable cm-scale patchiness of DFAA concentrations in the upper epilimnetic layer of lakes during the phytoplankton growth season, as predicted by the concept of DFAA release from macroscopic point sources. This is likely due to rather exotic requirements for dedicated sampling devices (Horrák et al. 2016) and the limitations of classic protocols (Lindroth and Mopper 1979) for high-throughput DFAA analysis.

While DFAA composition can vary between and within aquatic ecosystems, it often parallels that of microbial proteins, with a prevalence of small aliphatic amino acids (AA) (Réeeck 1983; Simon and Azam 1989). At the same time, selective hydrolysis may lead to differences between the DCAA and DFAA pools (Simon 1998). Concentrations of individual AAs in lake water are typically in the low nM range (Jørgensen and Søndergaard 1984; Simon 1998; Horňák et al. 2016) due to high microbial affinity (Fuhrman and Ferguson 1986). However, the coupling between consumption and release (Fuhrman 1987) may not be equally tight for different AAs (Horrák and Pernthaler 2019). Assuming spatially explicit macroscopic point sources of DFAA release (Grossart and Ploug 2001; Kiorboe and Jackson 2001; Simon et al. 2002), this allows for two scenarios: On the one hand, DFAA may be predominantly released from one type of source, e.g., peptide hydrolysis (Rosenstock and Simon 2001; Simon 1998), or microbial DFAA consumption may be non-selective (Egli 2010; Horňák and Pernthaler 2019). In this case, the composition of DFAAs should be similar in “hotspots” and at “background” concentrations, and DFAAs would be distributed mainly by small-scale physical mixing processes (Wüest and Lorke 2003; Taylor and Stocker 2012). DFAA composition would also be expected to more closely match to that of DCAAs. On the other hand, some AAs may be disproportionately released from specific particle types (Ovie and Ovie 2006; Sarmento et al. 2013), or free-living bacteria (i.e., not associated with the point sources) may preferably consume particular AAs during periods when overall DFAA consumption equals or exceeds release (Salcher et al. 2013; Horňák and Pernthaler 2019). These AAs would then be over- or underrepresented in release “hotspots” and “background” areas, respectively.

We investigated if cm-scale patches of significantly elevated DFAA concentrations were common in the upper epilimnetic layer of oligomesotrophic Lake Zurich, as predicted by the “macroscopic hotspot” concept of DFAA release. Fourteen sets of 100 water samples were collected at cm distances at different seasons over a time span of >3 years. The observed spatial patterns of the 15 most common AAs led us to test the additional hypothesis that AAs overrepresented in samples with peak DFAA concentrations were also preferably incorporated by the bacterioplankton.

**Materials and Methods**

**Lake sampling**

Fourteen sampling campaigns were conducted in the large, mesotrophic, prealpine Lake Zurich (Switzerland) (Bossard et al. 2001) between October 2016 and March 2020 (Supporting information Table S1). Water samples were collected near the deepest point (47°19’21”N, 8°33’42”E). A custom-built sampling device (Fig. 1) was used to assess small-scale spatial variability of DFAA concentrations at two spatial scales (cm to m). The device was designed to float in a horizontal position at 5 m depth by means of an attached buoy. Ten adjacent water samples were drawn simultaneously at a distance of 2 cm by vacuum through capillaries into 10 mL syringes that remained on board. The content of the syringes was then transferred into sterile 15 mL falcon tubes. The sampling procedure was repeated 10 times. Intervals between consecutive sample sets was on average 5 min. The volume of the capillaries was 10 mL, so that each sample except for the first one represented the content of the capillaries from the previous haul. In order to remove potential contamination within the apparatus three sets of lake water samples were drawn and discarded prior to the actual sample collection. The capillaries of the apparatus were stored in 70% ethanol between campaigns to avoid microbial growth, and thoroughly rinsed with ultrapure water (MQ-water, Direct 8 system, Millipore) prior to sampling. New sterile syringes were used for each sampling campaign. In the context of a parallel lake monitoring program, additional water samples were collected from the same depth with a 5 L-Friedinger sampler (Uwitec, Austria), and transferred into a 1 L glass bottle (Schott, Germany). All samples were transported to the laboratory at in situ temperature in the dark and processed within 1 h.

**Chl a concentrations and bacterial abundances**

The chlorophyll *a* (Chl *a*) concentrations of different phytoplankton groups were measured in situ with a TS-16-12 fluoroprobe (bbe Moldaenke, Kronshagen, Germany). Temperature profiles were estimated with an YSI multi-parameter probe (6600 YSI, Yellow Springs, Ohio). Bacterial abundances were determined from formaldehyde-fixed 2 mL subsamples (final concentration, 2%) obtained during the lake monitoring program. These samples were stained with SYBR green (Sigma-Aldrich, Saint Louis, Missouri) in DMSO (final concentration, 5%) and analyzed by flow cytometry (CytoFLEX, Beckman Coulter, Brea, California). To determine the total number of cells the resulting scatter plots of SYBR fluorescence intensity vs. side scatter were analyzed with the software CytExpert (Beckman Coulter).
Laboratory test of the sampling device

To establish the baseline variability of our sampling device, another 5 L lake water sample was collected in January 2021. Two liters of this sample were pre-filtered with a hollow fiber filtration apparatus (polysulfone capillary module 433 SEM-D-PS-AN-D-8A, 0.1 μm pore size Hydac Filtertechnik) and placed in a glass bowl that had been acid-washed and pre-rinsed with ultrapure water. Three sets of 10 samples were collected from this filtrate with the syringe sampler for subsequent DFAA analysis.

We also tested if there was a difference in DFAA concentrations between a set of 10 samples that were collected with the syringe sampler without any type of prefiltration and 10 samples that were manually collected with syringes fitted with a filter (IC Acrodisc, 0.2 μm Supor membrane, PALL Life Sciences). For this purpose, the glass bowl was filled with 2 L of unfiltered lake water and sampled accordingly.

High-performance liquid chromatography and mass spectrometry

One milliliter subsamples were filtered through syringe polyethersulfone filters (IC Acrodisc, 0.2 μm Supor membrane, PALL Life Sciences). Filters were pre-washed with 1 mL ultrapure water. DFAA were analyzed by high-performance liquid chromatography (1260 Infinity series, Agilent Technologies, Santa Clara, California) paired to a triple quadrupole mass spectrometer (API5000, AB Sciex) with an electrospray ionization source. Separation of DFAA was achieved on a YMC-Triart C-18 column (150 x 3 mm, 3 μm particle size, YMC) following the procedure described previously (Horňák et al. 2016), and using γ-aminobutyric acid (Gaba) as an internal standard. Subsequent DFAA detection based on compound specific pairs of precursor and product ions was performed according to the optimized method described by these authors (Horňák et al. 2016). Quantification of DFAA concentrations was done with the Multiquant software (version 2.1, AB Sciex). Separate calibration curves were made for all DFAAs that were included in the analysis, i.e., L-alanine (Ala), L-arginine (Arg), L-asparagine (Asn), L-aspartic acid (Asp), L-glutamine (Gln), L-glutamic acid (Glu), L-glycine (Gly), L-histidine (His), L-hydroxyproline (Hyp), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-ornithine (Orn), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), taurine (Tau), L-threonine (Thr), L-tryptophane (Trp), L-tyrosine (Tyr), and L-valine (Val) (all from Sigma–Aldrich at > 98% purity grade).

Variability of DFAA in 0.1 μm prefiltered lake water

DFAA concentrations in the 30 test samples drawn from 0.1 μm prefiltered lake water were first normalized to their median value. The normalized concentrations were then fitted to a number of theoretical distributions, using Kolmogorov–Smirnov and Anderson–Darling tests to assess goodness of fit (EasyFit 5.5, MathWave Technologies). The distribution that scored highest in both tests was selected to assess the variability of DFAA concentrations in lake water (normalized to the respective median values per sampling campaign). Specifically, we determined the number of samples in each campaign that had median-normalized DFAA concentrations outside the...
99.9% confidence intervals of the chosen distribution (i.e., the lowest and highest 0.005%), using the empirically derived values from the test samples to parameterize the corresponding probability density function.

Variability of DFAA composition between samples

The variability of DFAA composition between all 100 samples from a single sampling campaign and between the 10 samples of individual sets was examined by calculating normalized Euclidean distances. In order to avoid artifacts, those AAs with consistently low concentrations close to their respective limits of quantification (0.5 nM) were excluded from this analysis (Arg, Met, Hyp, Tyr, Trp, Phe, and Tau). Of the remaining 15 “common” AAs (collectively referred to as c-DFAA), the Euclidean distances between all 100 samples of a single sampling campaign were calculated using the “vegan” R package and default settings (R version 4.0.0) (Oksanen et al. 2016). For normalization, we used the proportional concentration of each AA in an individual sample relative to the c-DFAA concentration in this sample. The median distances per sampling campaign were then compared to the corresponding log-transformed median values of total DFAA concentrations by regression analysis. To assess the variability within individual sets of 10 simultaneously collected samples, normalized Euclidean distances were calculated only between samples from single sets, resulting in 10 per-set median distances for each sampling campaign. The averages of these 10 distances were then regressed on the log-transformed medians of DFAA concentrations per sampling campaign.

We also investigated if there were differences in c-DFAA composition between samples with DFAA concentration peaks and samples with “background” DFAA concentrations. For this purpose, we calculated the differences in the average relative contribution of individual AAs to c-DFAA between samples with the 10 highest and the 20 lowest DFAA concentrations per data set.

Incorporation of radiolabeled amino acids

Three incorporation experiments were conducted with samples collected on 15 August 2019, 1 October 2019, and 5 March 2020 in order to test if bacteria preferably consumed pulses of those AAs that had more spatially heterogeneous in situ availability, as compared to others that were always only present in low proportions.

Incubations were carried out using four radiolabeled amino acids: L-[14C]-serine (14C-Ser, specific activity 156 mCi mmol⁻¹, Moravek), L-[14C]-aspartic acid (14C-Asp, 208 mCi mmol⁻¹, American Radiolabeled Chemicals), L-[14C]-leucine (14C-Leu, 328 mCi mmol⁻¹, American Radiolabeled Chemicals) and L-[14C]-isoleucine (14C-Ile, 287 mCi mmol⁻¹, American Radiolabeled Chemicals). Amino acids were selected according to their concentrations in Lake Zurich waters (high: Ser, Asp; low: Leu, Ile) and their respective proportions in samples with high and low DFAA concentrations, as determined during several previous sampling campaigns.

Each set of 10 samples was used for incubations with one amino acid. The order of samples within a set were randomized. Four replicate samples (volume, 7 mL) were supplemented with the respective radiolabeled tracer (final concentration 20 nM) and incubated for 2 h in dark at in situ temperature. Four more replicates from the same set were amended with a combination of the radiolabeled tracer (final concentration 20 nM) and 50 times higher concentrations of the same, albeit unlabeled, amino acid (final concentration 1 μM). The last two samples served as controls, to which radiolabeled tracer was added after 30 min of fixation time (formaldehyde, 2% final concentration).

After the 2 h incubations, samples were fixed with formaldehyde (2% final concentration), filtered on mixed cellulose ester filters (GSWP, 0.2 μm pore size, Ø 25 mm, Millipore) and processed as described previously (Kirchman 2001). Briefly, the air-dried filters were put into scintillation vials and dissolved overnight in ethyl acetate. Subsequently, scintillation cocktail (Rotoszint eco plus, LSC-Univentalcocktail, Roth) was added and radioactivity (disintegrations per minute) was measured with a scintillation counter (Liquid Scintillation Analyzer, Tri-Carb 3170TR/SL, Perkin Elmer). Radioactivity incorporated into biomass was adjusted to abiotic adsorption of the radiolabel in fixed controls and converted into incorporation of the corresponding amino acid.

On one occasion (20 August 2019), we additionally explored if incorporation of the four amino acids could mainly be attributed to free-living bacterioplankton or to particle-associated cells. For this purpose, a water sample collected with the 5 L sampler was either processed directly or pre-filtered through 5 μm pore size filters (Whatman Nuclepore) prior to the incubations. Both variants were then split into 40 portions of 7 mL and incubation experiments were carried out as described above.

Results

Precision of total DFAA measurements and effect of prefiltration

The average concentration of total DFAAs in the 30 test samples with 0.1 μm prefiltered lake water was 216 ± 9 nM (mean ± 1 standard error). The median-normalized concentrations of the test samples was best modeled by a log-logistic 3-parameter distribution (subsequently referred to as “test distribution”; Goodness-of-Fit: Kolmogorov-Smirnov: D = 0.0784, p = 0.986, Anderson-Darling: $A^2 = 0.199$). The lower and upper limits encompassing 99.9% of values of the corresponding probability density function (α = 4.1724, β = 0.4899, γ = 0.5024) were 0.58 and 3.53 times the median concentration. These limits were selected as conservative thresholds to assess the extent of “real” DFAA variability in lake water in the field sampling campaigns.
The DFAA concentration of 10 test samples drawn in January 2021 with the syringe sampler from unfiltered lake water and without prefiltration was 171.3 ± 6.7 nM (mean ± 1 standard error). Ten test samples collected individually with syringes fitted with 0.2 μm filters had an average DFAA concentration of 173.6 ± 6.7 nM. There was no significant difference between either set (Mann–Whitney test, \( U = 42, p > 0.05 \)).

Chl \( a \) concentrations and bacterial abundances

Chl \( a \) concentrations at the sampling dates varied between 0.7 and 12.6 μg L\(^{-1}\) (Fig. 2a). With one exception (1 October 2019), the largest part of total Chl \( a \) could be assigned to the filamentous cyanobacterium *Planktothrix rubescens* (early spring and autumn), and to diatoms (late spring and summer). *P. rubescens* is the dominant primary producer in Lake Zurich; during the summer period, its distribution is largely limited to the metalimnetic zone (10–15 m depth) (Van den Wyngaert et al. 2011; Posch et al. 2012). There was no significant relationship between the Chl \( a \) concentrations of total phytoplankton, of *P. rubescens*, or of diatoms, and median DFAA concentrations. However, if one sampling campaign was excluded where diatoms formed a substantial spring bloom (9 May 2018, Fig. 2a), the fraction of non-*P. rubescens* derived Chl \( a \) was negatively correlated with DFAA variability (i.e., the number of samples with DFAA concentrations outside the limits of the test distribution; Pearson’s \( r = -0.68, p = 0.01, n = 13 \)).

Bacterial abundances generally ranged between 1.5 and 4.0 × 10\(^6\) cells ml\(^{-1}\) (Fig. 2b), with two conspicuous outliers (7 June 2017 and 15 March 2018). There was no significant correlation between bacterial abundances and any other parameter.

Variability of total DFAA concentrations in lake water

The median DFAA concentrations and their ranges greatly varied across all 14 sampling dates, and there was no apparent seasonal pattern in consecutive years (Fig. 2c). On average, only 53% (25–77%) of the 100 individual samples per campaign ranged between half and twice the overall median values. The highest 10% of DFAA concentrations per sampling campaign exceeded the lowest 10% by 5 to >200 fold (median, 20 fold). DFAA concentrations were always substantially skewed to the lower values (skewness: 4.0 ± 2.2, mean ± SD), and the medians of the lowest 10% of all concentrations per sampling accurately predicted the overall medians (linear regression, \( r^2 = 0.95, n = 14, p < 0.001 \)).

We compared the DFAA concentrations from lake water samples collected directly from the lake with the modeled distribution of concentrations in 30 replicate samples from 0.1 μm prefiltered lake water. Exactly one third of all samples collected directly from the lake had median-normalized DFAA concentrations outside the 99.9% confidence limits of the test distribution. On average, three such “outliers” were present in 127 of the altogether 140 sets of 10 simultaneously drawn samples.

The lowest variability was observed on 21 November 2017 (seven of 100 samples) and the highest on 21 March 2019 (56 of 100 samples). A significantly higher proportion of samples were below (23.3%) than above (10.1%) the critical limits of the test distribution (Wilcoxon Signed-Rank Test, \( z = -3.233, p < 0.01 \)), and the numbers of samples per campaign that were above and below these critical limits was...
significantly related (Pearson’s $r = 0.60, p = 0.02, N = 14$). There was no relationship between the number of “outlier” samples and the overall median DFAA concentrations per sampling campaign.

The median DFAA concentrations among sets of 10 simultaneously drawn samples varied by a factor of 5.4 (range, 1.3–26). There was a significantly negative relationship between the variability (CVs) of DFAA concentrations among sets and the overall median concentrations per sampling campaign (Spearman’s $\rho = -0.62, p = 0.018, n = 14$; Supporting information Fig. S1a). The CVs of DFAA concentration within single sets were significantly (1.5 to 4.5 times) higher than the CVs among sets (Mann–Whitney U Test, $p < 0.001, N = 14$).

Moreover, the within-set and among-set variability was significantly related (Pearson’s $r = 0.74, p = 0.002, N = 14$; Supporting information Fig. S1b). There was no significant difference between the sets that were collected first (i.e., without 5 min residence time in the capillaries) and the subsequent ones (Kruskal–Wallis ANOVA on median values per set and campaign, $N = 140, p = 0.48$).

Almost half (46%) of all pairs of successively collected sets of samples differed by < 20% in median concentrations, indicating that they originated from larger coherent spatiotemporal distribution patterns. Such patterns were apparent in the majority of sampling campaigns, e.g., as a continuous rise or drop of median DFAA concentrations across three or more sets, or as a systematic difference in concentrations between sets collected at the beginning or towards the end of the sampling campaign (Supporting information Fig. S2).

**Variability in DFAA composition**

The five most common amino acids together constituted on average 77% of total DFAA concentrations (Fig. 3). Ser and Asp formed a major fraction of total DFAAs in all samples, followed by Gly in all samples but one (24 April 2017). These three amino acids on average represented 60% of the DFAA pool. In addition, Ala was present in all except two samples, contributing on average 10% to total DFAAs. Other quantitatively important amino acids were Glu, Orn, Pro, Thr, and Gln, together forming on average 7% of total DFAAs.

There was a highly significant nonlinear relationship between total DFAA concentrations per sampling campaign and the compositional similarity (i.e., normalized Euclidian distances) of the 15 most common AAs (c-DFAA) in individual samples, as reflected by a logarithmic regression with a coefficient of determination of 0.77 (Fig. 4a). The slope of this regression was negative, i.e., the c-DFAA composition of individual samples became more similar at increasing DFAA concentrations. An equally strong relationship between overall median DFAA concentrations per sampling campaign and the median Euclidean distances between the c-DFAA composition of the samples was also observed at the scale of single sets of 10 samples that had been simultaneously obtained at 2 cm distances (Fig. 4b).

Based on these observations on overall composition, we proceeded to examine the differences in the relative contribution of individual AAs in samples with either “peak” or “background” DFAA concentrations, limiting ourselves to cases where these differences exceeded 10% of total c-DFAA
concentration. The three quantitatively most important amino acids Ser, Asp, and Gly (Fig. 3) repeatedly formed substantially higher proportions in the 10 samples with the highest total DFAA concentrations than in the 20 samples with the lowest concentrations (Fig. 5). Ser and Asp were overrepresented in five of 14 samplings, on average contributing 17% and 14% more to c-DFAA in "peak samples", respectively. Gly was overrepresented in 4 data sets, with an average difference of 14%. A disproportionately higher contribution of Thr (+11%) in samples with peak DFAA concentrations was detected once, on 21 March 2019, which was also the date when all other above listed amino acids were overrepresented by > 10%. Contrariwise, Gln, Lys and Tau were underrepresented in samples with peak DFAA concentrations, with Lys representing > 30% less in such samples on 21 March 2019 (Fig. 5, lower panel). As predicted by the relationship between overall DFAA concentration and the compositional variability of c-DFAA (Fig. 3), no concentration-dependent over-, or underrepresentation of single AAs was detected in sampling campaigns with high or intermediate overall DFAA concentrations (Figs. 2c, 5).

Incorporation rates of single AAs

Ser and Asp were among the most abundant AAs in all data sets (Fig. 3), and most frequently formed a disproportionally high fraction of c-DFAA in samples with peak DFAA concentrations (Fig. 5, upper panel). Therefore, we compared the incorporation rates of these two amino acids with those of a commonly used proxy for bacterial biomass production (Leu) (Kirkman 2001; Simon and Azam 1989) and a structurally similar one (Ile) that both had consistently low concentrations in Lake Zurich waters. On the three dates when the experiments were performed, Ser and Asp exhibited average concentrations of 4 nM and 2 nM, and peak concentrations of 18–42 nM and 12–18 nM, respectively, within the 40 samples used for the incubations. By contrast, Leu and Ile were present at concentrations < 1 nM at all three dates (Fig. 6a).

If only radiolabeled tracers were added, the highest incorporation rates were observed for 14C-Ser at all three seasons, followed by 14C-Asp (Fig. 6b). Conspicuously low
incorporation rates of 14C-Leu and 14C-Ile were found in the March experiments, in stark contrast to those of the other two tracers (Fig. 6b). Non-parametric Kruskal–Wallis ANOVAs (n = 4) indicated that the incorporation rates between individual amino acids significantly differed in all three experiments. 14C-Ser incorporation was always significantly different from both, 14C-Leu and 14C-Ile incorporation (Dunn’s post-hoc test, p < 0.05). 14C-Asp incorporation was significantly different from 14C-Ile incorporation in August and October and from both, 14C-Leu and 14C-Ile incorporation, in March (p < 0.05). The saturating pulse of unlabeled amino acids generally resulted in an increase of projected incorporation rates – compared to those at 20 nM by a factor that ranged between 2.3 (Ser, Oct) and 10.5 (Ile, March) (average, 5.1), with no systematic differences between individual amino acids (Fig. 6c).

In August 2019, we compared the incorporation rates of the four amino acids in unfiltered samples (as described above) and in 5 μm pre-filtered samples collected with a 5-L sampler. The incorporation rates of radiolabeled tracers in the pre-filtered samples ranged between 78% (Leu) and 94% (Ile) of those in the unfiltered samples (mean, 85%), and the respective differences in microbial uptake of the four amino acids closely resemble the pattern depicted in Fig. 6b (data not shown).

Discussion

Horizontal variability of DFAA concentrations

Our study provides first comprehensive field evidence that the DFAA substrate field in the upper epilimnion of an oligomesotrophic lake is highly variable at spatial scales of cm to m during the growing season of the phytoplankton (Fig. 2a,c). DFAA concentrations during a single sampling campaign typically varied by more than two, and up to four orders of magnitude, which is as high as or higher than the overall seasonal variability observed in bulk samples from lakes (Jørgensen 1987; Rosenstock and Simon 2001; Simon 1998).

Our sampling design assessed, both, the variability within single sets of 10 samples simultaneously drawn at distances of 2 cm, and the variability among 10 such sets collected from the same sampling location at intervals of approximately 5 min. Three patterns emerge from these data: (i) Heterogeneity at the cm-scale was common: samples with “outlier” DFAA concentrations (according to our distribution modeling) were found in > 90% of the collected sets. (ii) The variability within and among sets was significantly related (Supporting information Fig. S1b). Both scales contributed to the overall spatial heterogeneity of DFAA concentrations, albeit to a different extent (the variability was consistently higher within than among sets). This suggests a spatially connected hierarchy of intensifying variability of DFAA concentrations from larger to smaller scales, likely extending into even smaller dimensions (Stocker 2015; Taylor and Stocker 2012). (iii) The variability among sets tended to be highest if the overall median DFAA concentrations were low (Supporting information Fig. S1a). Moreover, the median values of successive sets of samples painted a clear picture of larger-scale
spatiotemporal patterns of DFAA concentrations (Supporting information S2).

All these findings appear to be consistent with the acting of turbulent mixing processes on DFAA distributions. Samples originated from 5 m depth, which is at the lower end of the energetic surface boundary layer, where wind-driven and convective turbulence shape horizontal and vertical transport at the scales of m and below (Wüst and Lorke 2003). This boundary layer seasonally changes together with overall vertical mixing processes, which might explain why DFAA concentrations were considerably more uniform during some sampling campaigns (e.g., November 2017, Fig. 1c). It should be noted that small-scale turbulent mixing and convective downwelling is important in the wave-affected surface layer of lakes, but does not extend into the interior water body, where wind energy flux is mainly channeled into basin-scaled waves (seiches) (Wüst and Lorke 2003). Therefore, our findings of DFAA variability in the surface boundary layer cannot be extrapolated over the whole water column or even the entire epilimnion.

Since we sampled in the typical layer of pelagic phytoplankton blooms in Lake Zurich (Van den Wyngaert et al. 2011; Neuenschwander et al. 2018), the observed variability likely also reflected biological processes. DFAAs may be released from various diffuse sources, e.g., DCAAs or proteins (Simon 1998; Rosenstock and Simon 2001), phytoplankton or bacterial exudates (Sarmento et al. 2013), viral lysis (Middelboe and Jørgensen 2006), and proto- and metazooplankton grazing (Riemann et al. 1986; Nagata and Kirchman 1991). The significant negative correlation between DFAA variability and Chl \(a\) concentrations (when excluding \(P.\) \textit{rubescens}\) and a pronounced spring diatom bloom) possibly hints at such diffuse release processes. On the other hand, DFAAs may be generated at point sources, e.g., by colonial algal or during degradation of particulate material from suspended organic aggregates (Grossart and Ploug 2001; Simon et al. 2002). On average, one in 10 samples per field campaign had significantly higher DFAA concentrations than expected by the technical variability of sampling (i.e., above the 99.9% confidence limits of our test distribution). Thus, our data provide field evidence for spatially explicit macroscale DFAA “hotspots” in the turbulent upper epilimnetic zone in Lake Zurich during different seasons, as predicted by the concept of DFAA release from macroscopic point sources (Kiorboe and Jackson 2001; Simon et al. 2002). Our sampling procedure was not designed to preserve delicate macroscopic aggregates, which often exceed the 1 mm diameters of the syringe nozzles and tubes that formed part of the sampling device (Grossart and Simon 1993; Simon et al. 2002). Therefore, it is plausible that some, if not all, of the sporadic peaks of DFAA concentrations (Fig. 1c) originated from the DFAA-rich pore water (Grossart and Simon 1998) of lake snow or other macroscopic organic particles that disintegrated during sampling.

DFAA composition

Our study agrees with previous reports that Ala, Ser, and Gly are dominant components of the DFAA pool in freshwater environments (Schürmann 1964; Jørgensen 1987; Simon and Rosenstock 1992; Fig. 3). In addition, Asp was one of the most important DFAAs in all our samplings over a 4-year period (Fig. 3). Asp was not detected at similarly high concentrations during a previous study in Lake Zurich (Höråk et al. 2016), and it was not compositionally relevant in Lake Constance (Simon and Rosenstock 1992) that shares many limnological properties with our study system. However, Asp has been described as an important element of the DFAA pool in Scandinavian lakes (Tranvik and Jørgensen 1995; Jørgensen et al. 1998).

We are not aware of a physical process that could have led to a contrasting spatial distribution of individual AAs in samples with high and low DFAA concentrations within a single sampling campaign. This finding is unlikely to be an artifact, because (i) we deliberately limited our analysis to the subset of AAs that could be reliably quantified in all samples, and (ii) the three most frequently overrepresented AAs in local DFAA “hotspots” were also dominant in lake water and did not pose a measurement challenge (Ser, Asp, and Gly, Figs. 3, 5).

There are at least two possibilities to explain our results from biological activities. On the one hand, it is conceivable that selective microbial consumption disproportionately reduced particular AAs at distances from the point source. This conclusion is supported by the observation that there was a surprisingly clear relationship between the spatial microheterogeneity of c-DFAA composition and median DFAA concentrations per sampling (Fig. 4): The compositional variability of c-DFAA between samples was distinctly more pronounced when median DFAA concentrations were only around 10–20 nM. On the other hand, the AA composition at the source might have been different from the “background” DFAA composition. Single algal species differ in their AA release patterns (Sarmento et al. 2013; Höråk et al. 2016), and so might algal (e.g., diatom) colonies. The dominant AAs in various zooplankton species (Ovie and Ovie 2006) or in particular tissues of single species (Schmidt et al. 2004) may greatly deviate from those in the typical DFAA pool. Of course, both explanations are not mutually exclusive. Degradation experiments with different types of particulate organic matter collected from Lake Zurich might help to elucidate the respective importance of source composition and selective consumption to the microheterogeneity of DFAA composition.

In either case, our results imply that (non-motile) planktonic bacteria may simultaneously face low overall DFAA concentrations and less predictable composition. This may force microbes to pursue a substrate generalist strategy (Matias et al. 2013; Sarmento et al. 2016) and to develop a “preparedness” for rare peaks of single substrate (Egli 2010). For example, filamentous \textit{Saprospiraceae} of the LD2 clade in spring
samples from Lake Zurich were extremely proficient in the uptake of N-acetylglucosamine (Eckert et al. 2013) even though this substrate is only available at sub-nM concentrations during that period (Horňák and Pernthaler 2014).

**Do AA incorporation preferences match with compositional microheterogeneity?**

We performed uptake experiments with several AAs to test the hypothesis that the observed differences in their small-scale horizontal distribution patterns (Figs. 4, 5) might be reflected in preferential microbial incorporation. Leu and Ile were chosen as representatives of spatially “inconspicuous” AAs that always formed similar proportions of c-DFAA in individual samples irrespective of total DFAA concentrations. Leu is also widely used to assess microbial biomass production (Kirchman 2001; Simon and Azam 1989). By contrast, the two other tested amino acids (Ser and Asp) had explicit spatial microheterogeneity in several sets of samples (Fig. 5).

Despite higher extracellular isotope dilution (Fig. 6a), the microbial assemblages in the epilimnion of Lake Zurich had significantly higher incorporation rates of Ser than of Leu or Ile, and higher uptake of Asp was also observed in two of three experiments (Fig. 6b). The community-level preference for peaks of Ser and Asp suggests that selective microbial consumption could have contributed to the proportional differences of these AAs between DFAA “hotspot” and “background” samples (Fig. 5). The increased incorporation rates at excess concentrations of the unlabeled compounds (Fig. 6c), moreover, indicates that pelagic microbes had the potential to respond to even higher peaks.

However, our data about the in situ availability of the tested AAs (Fig. 6a) should caution against an overly simplistic comparison of their uptake rates. The added amount of radio-labeled Ser and Asp (20 nM) was similar to their in situ peak concentrations, as observed within 40 samples (Fig. 6a). Therefore, Ser incorporation rates likely reflected a largely unbiased ecophysiological response of planktonic microbes to Ser “hotspots”, as might e.g., be encountered in the solute plumes around organic aggregates (Kiorboe and Jackson 2001). However, Leu was never present in lake water at more than sub-nM concentrations (Fig. 6a). The observed Leu incorporation rates at tracer concentrations of 20 nM (Fig. 6b) should thus be interpreted as reflecting the “hungry” nutritional state of planktonic bacteria (Ferenci 2001) and their metabolic “preparedness” (Egl 2010) to respond to excess amounts of this substrate that are never encountered in situ. Such utilization might not be physiologically equivalent to microbial in situ processing of Leu, e.g., a higher proportion of the compound might be respired if offered at 20 nM (Jørgensen 1987; Horňák and Pernthaler 2019) than at ambient concentrations (Ferguson and Sunda 1984; Hill et al. 2013). This casts doubt on the conclusion that higher rates of visible Ser incorporation (i.e., of the non-respired fraction of total uptake) were an unambiguous indication for uptake preference. More precise kinetic measurements (Jørgensen and Søndergaard 1984; Fuhrman and Ferguson 1986; Bertilsson et al. 2007) might be required to better assess the response of bacterioplankton to sudden surges of more or less spatially variable AAs within the DFAA pool.

While the differences in the bulk incorporation rates of Ser, Asp, and Leu, by and large, agreed with earlier data from Lake Zurich (Salcher et al. 2013), it is difficult to say which microbes might have been responsible for the observed rapid uptake of Ser and Asp. In an earlier study in Lake Zurich, only a negligible fraction of bacterial cells, but virtually all individuals of the filamentous cyanobacterium *P. rubescens* visibly incorporated these tracers (Salcher et al. 2013). However, *P. rubescens* was absent or had low abundances in the sampled depth layer during two of the three incorporation experiments (August, October 2019, Fig. 2a). Moreover, a 5 μm prefiltration—arguably removing most *P. rubescens* filaments—did not change the Ser and Asp uptake patterns. It is conceivable that the abundant picocyanobacteria in the summer plankton of Lake Zurich (Van den Wyngaert et al. 2011) might have played a role in Ser uptake, as demonstrated for *Synechococcus* in pure culture (Döhler 1982).

**Conclusions**

The majority of the bacterioplankton in the upper epilimnion of Lake Zurich are nonmotile cells, e.g., affiliated with the genera *Candidatus* Nanopelagicus, *Candidatus* Fonsibacter, *Polynucleobacter*, *Limnohabitans*, or *Flavobacterium* (Salcher et al. 2011; Eckert et al. 2013; Neuen Schwan der et al. 2018). The observed heterogeneity of DFAA concentrations (Fig. 2c) or composition (Figs. 4, 5) in the surface boundary layer implies that these taxa do not experience uniform conditions, but exist within a distinct cm- to m-scale substrate “landscape.” From a metapopulation perspective, individual “local” populations (of 10⁴ or more cells) of such nonmotile bacteria are thus separated into zones of more or less favorable growth conditions by turbulent mixing processes. In order to better understand, the specific ecophysiological adaptations of these bacteria it may be important to also consider their variable natural environment.

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