Homeostatic regulation of dissolved labile organic substrates by consumption and release processes in a freshwater lake

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Abstract

We examined short-term changes and spatial variability of dissolved free amino acids (DFAA) and glucose in mesotrophic Lake Zurich (Switzerland) at 2- to 4-day intervals over 5 weeks during spring. Heterotrophic incorporation, consumption (“gross” uptake), and release of free leucine and glucose were assessed using isotopically labeled tracers. Glucose exhibited significantly higher consumption and release rates but a lower turnover time than leucine. The net uptake, release, and turnover time of both substrates were significantly correlated, suggesting that their microbial processing was closely coupled. Net changes of individual DFAA and glucose during dark short-term incubations with unfiltered samples including phytoplankton were conspicuously similar, indicating that bulk heterotrophic consumption of labile substrates was nonselective and depended primarily on their availability. Changes of DFAA during incubations were significantly related to their initial concentrations. Specifically, DFAA concentrations tended to converge toward their seasonal in situ median concentrations, which likely represented the balanced state of net release vs. consumption. There was a clear difference between relatively stable compounds (glucose, leucine, arginine) and others that showed pronounced concentration changes during incubations (alanine, valine, glycine), pointing to a variable degree of uncoupling between consumption and release. Moreover, short-term changes of DFAA concentrations in incubations were also related to their variability in replicate 5-liter samples taken from the lake, suggesting that biological processes such as consumption and release may influence the mesoscale spatial distribution of DFAA in the epilimnion of a lake. Overall, we document the homeostatic regulation of labile dissolved substrates by varying release and consumption processes.

Labile low-molecular-weight (LMW) compounds only constitute a minute fraction of the dissolved organic matter in aquatic systems (Søndergaard and Middelboe 1995), yet the uptake of these organic monomers may account for the majority of carbon and energy acquired by heterotrophic microbes (Kirchman 2003). From the wide array of LMW compounds considerable attention has focused on the dissolved free amino acids (DFAA) and carbohydrates (DFCH) because these compounds could be readily identified and quantified (Lindroth and Mopper 1979; Mopper et al. 1992). Their uptake can satisfy most if not all of bacterioplankton biosynthesis and energy demands, and they are therefore considered important for fueling the microbial food web (e.g., Rosenstock and Simon 1993; Bunte and Simon 1999). Ambient concentrations of individual DFAA and DFCH in the water column are typically at the low nM levels, which contrasts with their rapid turnover and large flux (i.e., the amount of DFAA and DFCH exchanged between different amino acid and carbohydrate pools) (e.g., Jørgensen and Jensen 1994; Rosenstock and Simon 2001).

The composition of the DFAA pool generally reflects that of planktonic proteins, with a dominance of small aliphatic forms (e.g., alanine, glycine) (Reeck 1983). Leucine is often used as a model for assessing overall amino acid uptake and flux, because it is thought to be incorporated by the majority of planktonic bacteria (Kirchman et al. 1985; Pérez et al. 2010). Nearly all proteinogenic and other important amino acids can be found in lake water, whereas the range of detectable DFCH compounds is much lower. Glucose typically dominates the total dissolved labile carbohydrates, with concentrations that are often equal or higher than the total of other DFCH and amino sugars, that is, between 5 and 20 nM (e.g., Jørgensen and Jensen 1994; Skoog et al. 1999; Horňák and Pernthaler 2014). In addition, glucose turnover and uptake rates are usually higher than those of other carbohydrates (Jørgensen and Jensen 1994; Rich et al. 1996; Bunte and Simon 1999). The rapid and efficient microbial utilization of DFAA and DFCH likely control their low ambient availability (Nissen et al. 1984). This process

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is counterbalanced by the amino acid and carbohydrate release via algal and bacterial exudation, enzymatic polymer degradation, grazing or viral lysis (Nagata 2000).

Although DFAA and DFCH occur at low nM levels, the ambient concentrations are highly variable at different temporal and spatial scales (Coffin 1989; Simon 1998; Meon and Jüttner 1999; Horňák et al. 2016), pointing to a potential uncoupling between the instantaneous consumption and release processes. Such uncoupling might arguably induce rapid changes in local concentrations of these LMW compounds at the scale of hours. However, the magnitude of such fluctuations in DFAA and DFCH concentrations is poorly understood. The short-term net changes of alanine concentrations in sea water were small (<30%) compared to consumptions and release rates (Fuhrman 1987), suggesting a tight control of this LMW compound. It is unclear whether this pattern holds true for DFAA in general or if individual amino acids differ in their extent of short-term temporal variability. Moreover, it is conceivable that such temporal fluctuations might contribute to the high spatial variability of DFAA concentrations at the mesoscale, as has been observed in the pelagic zone before (Horňák et al. 2016).

We analyzed the temporal changes in the concentrations of DFAA in the pelagic zone of the large prealpine mesotrophic Lake Zurich (Switzerland) determined at two levels of resolution (h, d) by a series of short-term incubation experiments performed over a 5-week period during the spring phytoplankton bloom. Due to short turnover times of DFAA, rapid changes in their ambient concentrations were assumed. By this we explored the hypothesis that the short-term temporal changes of individual DFAA resulting from the balance between their heterotrophic utilization and release may be dependent on their ambient concentrations. In parallel, we simultaneously measured the incorporation, consumption, and release rates of leucine and glucose by planktonic microbes to examine their importance in supporting the biosynthesis and energy requirements and recycling of these labile molecules. Finally, we tested if the short-term temporal changes of DFAA concentrations over several hours might be related to the observed variability of their mesoscale spatial distribution.

**Materials and methods**

**Sampling and environmental parameters**

Lake Zurich (406 m above sea level, area 65.06 km², max. depth 136 m, mean depth 51.7 m, residence time 440 d, Switzerland) is a large oligo-mesotrophic prealpine lake (Bossard et al. 2001). A site located 500 m offshore of the Limnological Station (47°19’N, 8°34’E) was sampled at intervals of 2–4 d from 20 March 2014 to 24 April 2014. Samples were taken between 9 am and 11 am from the depth of 5 m with a Friedinger sampler (diameter 14 cm, volume 5 L, integrated depth 33 cm, Uuwtec, Austria). Samples were collected into acid-washed glass bottles (volume 1 L) and kept for 30 min at in situ temperature in the dark until further processed. In situ chlorophyll a concentrations corresponding to different phytoplankton groups (diatoms, cryptophytes, green algae, cyanobacterium *Planktothrix rubescens*) were measured with a multiple wavelength probe (TS-16-12 fluoroprobe, bbe Moldaenke, Germany). Temperature was determined using a multiparameter probe (6600, YSI). Both probes were calibrated prior to each sampling.

**Liquid chromatography and mass spectrometry**

DFAA and DFCH were analyzed by high-performance liquid chromatography (1260 Infinity series, Agilent Technologies) coupled to a triple quadrupole mass spectrometer (API5000, AB Sciex, Canada) applying electrospray ionization. Compound-specific pairs of precursor and product ions were used to detect the analytes. Briefly, DFAA were separated on a reversed-phase column (Uptisphere X-Series C-18, 50 × 2.1 mm, particle size 3 μm, Interchim, France) using tridecafluorohexanoic acid as a ion-pairing reagent (Horňák et al. 2016). Concentrations of DFCH were determined by ion-exclusion chromatography on a cation exchange column (Supelcogel C-610H, 300 × 7.8 mm, particle size 9 μm, Supelco) employing a gradient elution with formic acid as a mobile phase (Horňák and Pernthaler 2014). The multiple reaction monitoring scan mode was used to acquire data (Analyst, version 1.6.1, AB Sciex). The integration of peak areas, including the quantification of 13C-labeled tracers, was performed with the MQ4 algorithm (Multiquant software, version 2.1, AB Sciex) using the appropriate internal standards determined independently in each sample. Detection limits of DFAA (Horňák et al. 2016) and DFCH (Horňák and Pernthaler 2014) ranged from 0.05 to 2 nM. All samples were filtered through a 0.2 μm syringe filter prior to analyses.

**Experimental incubations with isotope-labeled leucine and glucose**

For bulk incorporation assays, triplicated live samples of 10 mL and one control prefixed with paraformaldehyde (PFA, final concentration 2%) were incubated either with [3H]-leucine (specific activity 60 Ci mmol⁻¹, American Radiolabeled Chemicals) or [3H]-glucose (specific activity 29 Ci mmol⁻¹, Amersham) for either 0.5, 1, 2, and 4 h at in situ temperature in the dark. Radio-tracers were added at a final concentration of 20 nM L⁻¹. Incubations were terminated by adding PFA (final concentration, 2%). Samples were then filtered onto GSWP membrane filters (pore size 0.2 μm, diameter 25 mm, Millipore). Disintegrations per minute in live samples determined with a scintillation counter (Tri-Carb 3170TR/SL, Perkin-Elmer) according to the ice-cold trichloroacetic acid extraction method (Kirchman 2001) were corrected for abiotic adsorption of radiolabeled tracers measured in killed controls and subsequently converted to leucine and glucose incorporation rates.

Total glucose and leucine consumption rates (i.e., “gross” uptake) were determined in triplicate samples of 1 L amended either with [U-13C₆]-leucine or [U-13C₆]-glucose (99%, Cambridge
Isotope Laboratories) at a final concentrations of 20 nmol L$^{-1}$ incubated at in situ temperature in the dark for 4 h. Duplicate controls without tracer additions were incubated in parallel. Subsamples of 5 mL taken at 0, 0.5, 1, 2, and 4 h of incubation were filtered through a polyethersulfone syringe filter (pore size 0.2 μm, diameter 13 mm, Pall) prewashed with MilliQ-water. Filtrates of 1 mL were collected into HPLC vials (volume 2 mL, Glastechnik Graefenroda, Germany) and kept at −24°C until further analyses.

Calculation of the consumption and release rates, retention efficiency and turnover time of leucine and glucose

Temporal changes in the concentrations of $^{13}$C-labeled (tracer) and nonlabeled (natural) leucine or glucose in experimental incubations were compared to obtain information on their flux based on the corresponding incorporation, consumption, and release rates. The total consumption ("gross" uptake) and total release (production) rates were determined according to an isotope dilution model (Blackburn 1979; Caperon et al. 1979), using the equations described in the supplementary material. Because the addition of tracers may change the substrate dynamics by substantially increasing substrate availability, the concentrations of nonlabeled glucose and leucine in treatments were compared with those in controls without tracer amendments. However, no significant differences in the concentrations of nonlabeled leucine and glucose were observed. The retention efficiency of leucine and glucose (i.e., the fraction of leucine and glucose incorporated into microbial biomass) was estimated from the differences between the incorporation rates measured by radioassays and the corresponding consumption rates measured with stable-isotope tracers. Turnover times of leucine and glucose were calculated as a quotient of the total consumption rates and the corresponding leucine and glucose concentrations, respectively.

Statistical analyses

For the analysis of the time series at 2- to 4-d intervals, a 2-way ANOVA (date, DFAA) was performed, followed by Scheffé post-hoc tests to identify groups of dates that significantly differed from each other. Changes in the DFAA concentrations after 4 h of dark incubation were subsequently expressed as log$_2$ ratio (i.e., DFAA concentration at T$_{4h}$/DFAA concentration at T$_{0h}$) and tested for significance by a $t$-test against a hypothetical mean value (i.e., zero). A positive ratio indicates net release of DFAA, whereas the opposite corresponds to net DFAA consumption. In addition, linear regression analysis was used to examine the relationship between the ambient DFAA concentrations (T$_{0h}$) and absolute changes in their concentrations after 4 h of dark incubation ($n = 44$). Furthermore, short-term variations in DFAA concentrations (as mean proportional changes in their concentration after 4 h of dark incubation) were compared with the corresponding spatial variability (as a coefficient of variation of four replicates taken individually at each sampling date).

Prior to the linear regression analysis, both spatial and short-term changes in DFAA were normalized using the Box-Cox transformation (Box and Cox 1964). Spearman’s correlation analysis was applied to test for synchronous changes in the concentrations of individual DFAA adjusted for the false discovery rate by the Benjamini-Hochberg procedure. Statistical analyses were performed using the programs Paleontological Statistics (PAST, http://folk.uio.no/ohammer/past) and SPSS (IBM).

Results

Temperature and chlorophyll $a$ concentrations

Temperature at 5 m gradually increased from 6 to 13°C during the studied period between March and April 2014, and total chlorophyll $a$ concentrations varied between 3 and 32 μg L$^{-1}$ (Fig. 1). The filamentous cyanobacterium $P.$ rubescens constituted...
80% of the total chlorophyll a concentrations, whereas diatoms dominated at the end of the sampling period (data not shown). Markedly elevated chlorophyll a concentration detected in April were due to an internal wave (seiche) which pushed metalimnetic water containing high biomass of *P. rubescens* up to a depth of 5 m.

**Concentrations and fluxes of glucose and leucine**

Concentrations of dissolved free leucine and glucose varied between 1–3 and 4–10 nmol L⁻¹, respectively (Fig. 2a). The variable in situ concentrations of glucose and leucine as well as the observed rapid changes in their concentrations after 4 h of dark incubation indicated imbalanced substrate fluxes, which was confirmed by short-term incubations with isotope-labeled tracers. The release and consumption rates of glucose and leucine typically ranged from 0.2 to 1.5 nmol h⁻¹ and were often uncoupled (Fig. 2b,c). The mean uptake and release rates of leucine were 0.5 nmol h⁻¹, while those of glucose were 1.0 and 0.8 nmol h⁻¹, respectively. Leucine net flux was positively related with glucose net flux (Fig. 2d, $R^2 = 0.61$, $n = 11$, $p < 0.05$), and changes in the net leucine flux were reflected by more than twice as high changes in the net glucose flux, as deduced from the slope of that linear relationship. In contrast, no significant relationships between the consumption rates and ambient concentrations of leucine and glucose were found. The turnover time of leucine varied between 1.6 and 8.6 h, while that of glucose ranged from 3.4 to 33.7 h (Supporting Information Fig. S1). On average, leucine turnover was more than twice as fast as glucose turnover during the studied period. The turnover time of both substrates were significantly correlated (Pearson’s $r = 0.69$, $n = 11$, $p < 0.05$) and increased with increasing ambient concentrations. Also the incorporation of leucine and glucose into biomass, estimated as fractions of gross uptake, were positively correlated (Pearson’s $r = 0.61$, $n = 11$, $p < 0.05$). Notwithstanding temporal variations between individual incubations over the study period, the mean incorporation rate of leucine into biomass (0.2 nmol h⁻¹) was higher than of glucose (0.13 nmol h⁻¹). This difference was more pronounced when incorporation rates were related to the total uptake rates: on average, 39% of total leucine (Supporting Information Fig. S1) but only 16% of glucose was incorporated into proteins (Supporting Information Fig. S1).

**Within season and short-term changes in DFAA concentrations**

Average in situ concentrations of the total DFAA pool ranged from 38 to 172 nmol L⁻¹, and they tended to be higher in the second half of the study period (Fig. 3a, 2-way ANOVA followed by Scheffé post-hoc tests): While the total DFAA concentrations usually did not exceed 50 nmol L⁻¹ during the first half of the sampling campaign (until 3 April), they were mostly between 70 and 90 nmol L⁻¹ thereafter. Concentrations of dissolved free amino acids fluctuated on a diurnal basis, with maximum values measured during the day and minimum values during the night (Fig. 3b, 2-way ANOVA followed by Scheffé post-hoc tests). Leucine, the isotope-labeled amino acid, was consumed at a rate of 3.9 nmol h⁻¹, while that of glucose was 1.9 nmol h⁻¹, which was more than twice as high as glucose turnover during the studied period. Within season and short-term changes in DFAA concentrations and fluxes of free glucose and leucine (determined as differences between the release and total uptake, as well as consumption and incorporation) are illustrated in Fig. 2.

**Fig. 2.** (a) Concentrations and (b) total consumption, release, and incorporation rates of dissolved free leucine and (c) glucose in epilimnetic samples (5 m) collected from Lake Zurich between March and April 2014. (d) Linear regression analysis between the net flux of leucine and glucose (determined as differences between the release and consumption rates) in the same data set ($n = 11$), $R^2 = \text{coefficient of determination.}$
most individual DFAA at different sampling days were significantly correlated within each other (Supporting Information - Table S1). Overall, the individual DFAA exhibited a relatively constant mol % value over the entire study period. Rapid changes in total DFAA concentrations were observed in the short-term incubation experiments with unfiltered lake water samples that included also phytoplankton (Fig. 3b). In March 2014 the concentrations of DFAA mostly increased, whereas the opposite trend was found in April 2014. Most importantly, the decreases or increases of total DFAA (Fig. 3c) as well as of individual amino acids were significantly related to their respective ambient concentrations; this relationship was not restricted to the quantitatively most important DFAA (Fig. 4a,b, Supporting Information Fig. S4). However, the ranges of short-term changes during dark incubations over the whole study period were very different for individual DFAA. For example, concentrations of alanine (Supporting Information Fig. S2), valine, glycine, or glutamic acid showed changes that were as pronounced as or higher than those of the total DFAA concentrations, whereas other DFAA, such as arginine (Supporting Information Fig. S2), leucine (Supporting Information Fig. S3),
or isoleucine, appeared to be rather stable during 4 h of dark incubations. Although the short-term changes in all individual DFAA were significantly related to their ambient concentrations, the corresponding coefficients of determination \(R^2\) and to a lower extent also the slopes of the linear regression varied greatly (Fig. 4b, Supporting Information Fig. S4). The short-term changes in concentrations of most DFAA, as exemplified by glycine or glutamic acid, could be well predicted by their in situ concentrations (resulting in a coefficient of determination >0.5, Fig. 4b). However, with decreasing \(R^2\) values, the changes in the DFAA concentrations, as exemplified by leucine or arginine, tended to be more stochastic and they did not deviate substantially from the median value over a period of several hours. This is interpreted as a difference in the coupling of release and consumption processes.

**Short-term vs. spatial variations in DFAA**

We compared the short-term concentrations changes of total DFAA after 4 h of incubation in the dark at in situ temperature (relative change compared to initial time point) with the spatial variations of four replicate hauls from the same sampling date (relative difference between highest and lowest concentration). The short-term temporal changes in DFAA concentrations significantly contributed to their spatial variability (Fig. 5).

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**Fig. 5.** Relationship between the short-term and spatial variations in total DFAA (individual DFAA pooled together), aspartic acid, glutamine and leucine in epilimnetic samples (5 m) collected from Lake Zurich between March and April 2014. Short-term variations correspond to the proportional changes in DFAA concentrations after 4 h of incubation in the dark at in situ temperature. Spatial variations are relative differences in DFAA concentrations between four replicates at T<sub>0h</sub> compared to the initial mean DFAA concentrations. Both parameters were normalized using the Box-Cox transformation. Coefficient of determination \(R^2\) was calculated with linear regression analysis.
Moreover, a significant relationship between these two parameters was also found for aspartic acid, glutamine and leucine (Fig. 5).

**Discussion**

Stable yet dynamic concentrations of free leucine and glucose

While the measured ambient leucine and glucose concentrations were very low (Fig. 2a), the consumption rates of both substrates were high (Fig. 2b,c), which is consistent with previous reports (Kirchman 2003). Despite the higher consumption rates of glucose leucine had a shorter turnover time than glucose (Rich et al. 1996; Weiss and Simon 1999), likely due to the substantially lower ambient leucine concentrations. Different proportions of the substrates were utilized for biomass synthesis and energy requirements, respectively. While nearly all of the glucose that was taken up was also respired (84%), a substantially larger fraction of leucine (39%) was used for biomass production (Fig. 2b,c). The percentage of incorporated leucine and glucose was positively correlated (Pearson’s r = 0.61, n = 11, p < 0.05,) and the substantially higher fluctuations in the proportions of biomass-retained leucine (Supporting Information Fig. S1a) might reflect the instantaneous state of microbial C metabolism at different sampling time points. A higher fraction of incorporated leucine implies higher protein synthesis, whereas a lower percentage suggests that leucine was predominantly catabolized in the citric acid cycle. Our results are in line with previous studies reporting a variable fraction of respired DFAA and DFCH (e.g., Jørgensen 1987; Jørgensen and Jensen 1994), but contrast earlier reports of stable proportions of respired amino acids and glucose in an estuary (Crawford et al. 1974).

The uptake rates of leucine and glucose were not related to their respective ambient concentrations, implying that the observed patterns of substrate dynamics could not be solely explained by consumption. By contrast, approximately 60% of the variability in glucose net flux could be explained by the net flux of leucine (Fig. 2d). This is evidence that both substrates underwent similar in situ dynamics, despite originating from distinct classes of labile organic matter. Thus, their ambient concentrations were likely controlled by comparable mechanisms, suggesting that general (e.g., uptake, grazing, lysis) rather than compound-specific (e.g., enzymatic degradation, exudation) processes played a central role in defining the concentrations of free leucine and glucose. Positive relationships between the fluxes, turnover times, and biomass retention efficiencies of leucine and glucose, moreover, suggest that both substrates were equally attractive for microbial consumers. These findings clearly contrast with the previously reported preferential utilization of amino acids (Kirchman 1990; Schweitzer and Simon 1995). However, the microbial processing of DFAAs and glucose (such as the size of the respired fraction) may vary seasonally in mesotrophic lakes (e.g., Weiss and Simon 1999). Since our data were solely collected during spring, it is conceivable that the observed coupling between the fluxes of leucine and glucose may be a season-specific phenomenon, which is also influenced by varying nutrient availability.

Short-term changes in DFAA

The in situ concentrations of DFAA varied between sampling dates (Fig. 3a) and were prone to massive fluctuations in short-term dark incubations (mean, 40% of initial concentrations, range, 6–92%, data from 11 independent experiments). These changes comprised both net consumption and net release, which were detected equally often and at comparable magnitudes (Fig. 3b). Our data illustrate a highly dynamic pattern of imbalance between the heterotrophic release and utilization of individual and total DFAA, and thus a transient rather than steady state of their ambient concentrations.

The incubations (Fig. 3b) repeatedly resulted in significantly increased concentrations of total DFAA (t-test against hypotheti-

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both heterotrophic consumption and release are balanced, in analogy with a negative feedback loop oscillating around a given equilibrium (e.g., Thomas and D’Ari 1990). Our study thus generalized earlier results of Fuhrman (1987) on alanine dynamics, and it goes beyond the findings of that study by showing that this feedback may vary across different DFAA: The short-term changes of some DFAA were tightly related to ambient concentrations (i.e., they changed in a predictable manner), whereas other DFAA changed less predictably, resulting in a lower—albeit still significant—coefficient of determination (Fig. 4b, Supporting Information Fig. S4). For instance, valine and leucine are structurally related (branched-chain amino acids), have similar uptake kinetics (Jørgensen 1992), and had comparable concentrations in our samples (Supporting Information Fig. S3a). However, their respective coefficients of determination and slopes of the linear regressions suggested fundamental differences between the patterns of short-term concentration changes (Supporting Information Fig. S4), that is, a more (valine) or less (leucine) predictable coupling between release and consumption processes (Fig. 4c). Thus, for DFAA with high coefficients of determination such as valine, there seems to be a quasi “homeostatic” feedback between their ambient concentrations in lake water and the magnitude and direction of their short-term changes within the 4-hour time window of our incubations. By contrast, other, more “stochastically changing” DFAA such as leucine either do not substantially deviate from the seasonal median value (i.e., are tightly regulated), fluctuate more randomly, or the relationship between their ambient and short-term concentration changes might depend on longer incubation periods. Moreover, as the short-term changes of glucose concentrations in dark incubation were also significantly related to its ambient concentrations (Fig. 4), our proposed model of short-term fluctuations of ambient concentrations around a given threshold might thus be valid for different classes of LMW compounds. Glucose belonged to the subset of compounds with a more “stochastic” coupling of release and consumption, comparable to leucine and isoleucine (Fig. 4), which may arguably reflect the uptake preferences of heterotrophic consumers.

It is conceivable that the ambient DFAA concentrations below the observed threshold (seasonal median) concentration of balanced release and consumption represent the “residual concentrations” that are too low to be rapidly taken up by the bacterial community. Indeed, the typical uptake rates of DFAA in freshwaters are substantially below (<10%) the maximal uptake rates ($V_{\max}$), as demonstrated previously for arginine (Bertilsson et al. 2007), leucine and valine (Jørgensen 1992). Therefore, uptake of DFAA appears to be primarily limited by their release; in fact, transport systems appear to be adapted to elevated DFAA concentrations (Jørgensen 1992; Bertilsson et al. 2007) that occur sporadically (Hornák et al. 2016). Possible factors that can lead to higher release of DFAA may include extracellular primary production, zooplankton excretion, cell lysis after viral infection or from a declining bloom (Lehman and Scavia 1982; Mitchell et al. 1985; Blackburn et al. 1998).

**Does planktonic biomass contain sufficient leucine to sustain its release?**

The plausibility of the observed fluxes of LMW compounds and their short-term net changes can be assessed by comparing them with the standing stock biomasses of planktonic microbes, as subsequently exemplified for leucine. Proteins account for 63% of cell dry weight in bacteria (Simon and Azam 1989) and for 44% in *P. rubescens* (Konopka 1982). *P. rubescens* dominated primary production (>80% of total chlorophyll a concentration) and total plankton biomass in our samples. The mean biovolume of planktonic bacteria ranged between 0.06 and 0.07 μm$^3$ cell$^{-1}$ (Posch et al. 2009) that corresponds to approximately 30 fg dry weight or 18.9 fg protein cell$^{-1}$. The fraction of leucine in bacterial protein is 7.3% (Simon and Azam 1989), yielding a per cell quota of 1.4 fg, which, multiplied by the average abundance of planktonic bacteria in our samples (1.9 × 10$^6$ cells mL$^{-1}$), corresponds to 2.62 μg or approximately 20 nmol leucine L$^{-1}$. The total biovolume of *P. rubescens*, based on microscopic measurements (Zeder et al. 2010), was on average 2.36 mm$^3$ L$^{-1}$, corresponding to 639 μg L$^{-1}$ of dry weight (Zotina et al. 2003). Leucine accounts for 4% of total protein in *P. rubescens* (K. Hornák, unpublished data, obtained from *P. rubescens* collected from Lake Zurich in spring 2018). Thus, the estimated amount of leucine in *P. rubescens* biomass was 11.2 μg or 86 nmol leucine L$^{-1}$. The mean release rate of leucine in the dark incubations was 0.5 nmol h$^{-1}$ (Fig. 2b), that is, <1% of the estimated leucine content of bacterial and *P. rubescens* biomass. Therefore, it appears plausible that the planktonic microbial biomass in the experimental incubations was sufficient for the observed supply of free leucine. We can only speculate about the amount of leucine released either enzymatically or via predation and cell lysis. However, it is conceivable that a fraction of leucine in bacterial biomass was channeled via grazing (e.g., by heterotrophic flagellates or ciliates) to higher trophic levels. In contrast, *P. rubescens* is resistant to grazing and releases only negligible amounts of exudates (Feuillade et al. 1990) suggesting that biomass of this cyanobacterium was a sink of leucine.

**Synchronized changes and nonselective consumption of DFAA**

The tight coupling between the net fluxes of leucine and glucose (Fig. 2d) together with the synchronized temporal changes of the majority of individual DFAA (68% of individual DFAA were significantly correlated with total DFAA concentrations, Pearson’s $r$ ranged from 0.72 to 0.99) as well as significant correlations within individual DFAA (Supporting Information - Table S1) seems to imply a nonselective consumption of labile substrates by the heterotrophic community where the uptake of individual substrates predominantly depends on their actual
concentration. The limiting availability of labile DOC components might favor microbes that are able to simultaneously utilize multiple substrates at very low concentrations (Ilsness and Egli 2005). Also, the synchronous patterns of the concentration changes of individual DFAA in the incubations (as, e.g., reflected by their significant relationships with ambient concentrations, Fig. 4b, Supporting Information Fig. S4) suggests that microbial utilization of the DFAA pool was not restricted to few preferred or dominant compounds. Instead, the majority of DFAA were simultaneously utilized, in agreement with the concept of nonselective microbial substrate utilization (“mixed substrate growth”) under oligotrophic conditions (Egli 2010). In this model, a simultaneous consumption would allow microbes to coassimilate substrates at lower concentrations than by focusing uptake on single substrates only (Lendenmann et al. 1996). Such a scenario seems advantageous for the utilization of DFAA available at low nM concentrations.

This conclusion appears to contrast with the concept of taxon-specific “target substrates”, as, for example, deduced from microautoradiography analyses (Buck et al. 2009; Eckert et al. 2012) examining individual cells or populations. However, the uptake of many labile substrates appears to be widespread across different taxa of pelagic bacteria (e.g., Riemann and Azam 2002; Krempaska et al. 2018). Thus, the coexistence of numerous populations of more or less specialized bacteria with overlapping substrate spectra (Salcher et al. 2013) would result in exactly the type of apparently “generalist” substrate consumption pattern at the community level as observed in our experiment.

Our short-term incubations were performed in the dark. Yet, it is well established that primary producers also contribute to the supply of LMW compounds via extracellular production (Hama and Handa 1987). Therefore, it does not necessarily follow from our observations that in situ DFAA concentrations should undergo similar short-term fluctuations also in the water column during the light hours of the day. However, the significant relationship between the short-term changes in DFAA concentrations (obtained from dark incubations) and their respective mesoscale spatial differences (assessed during daylight hours) (Fig. 5) as discussed below suggests that the here proposed model might nevertheless be of relevance for the realized substrate distribution patterns in lake water.

**Mesoscale spatial variability: Consequence of imbalanced consumption and release of DFAA?**

The concentrations of dissolved organic matter (including labile organic compounds) spatially vary in pelagic environments (e.g., Stocker 2012). At the smallest scale, these variations result from sudden bursts of organic matter released extracellularly or after cell lysis (Mitchell et al. 1985; Blackburn et al. 1998), zooplankton excretion (Lehman and Scavia 1982) and leaking from particles (Kiorboe et al. 2001). Together, these mechanisms create a heterogeneous environment with ephemeral patches containing elevated concentrations of organic matter at the scale of micrometers to centimeters (Stocker et al. 2008). In addition, physical structuring of the surface water column may occur at scales of centimeters to meters, for example, due to turbulent mixing (Wüest and Lorke 2003) or thermal stratification. Such processes might in fact translate the patchiness of substrate concentrations that is biologically generated at a microscale into larger patterns of heterogeneity (Horňák et al. 2016).

We observed that the mesoscale spatial heterogeneity (at the scale of meters) of pooled DFAA and of some individual DFAA (aspartic acid, glutamine, leucine) was significantly related to their short-term temporal variations during dark incubations (Fig. 5) resulting from the unbalance of consumption and release rates. Our results suggest that besides physical factors, biological processes potentially also contribute to the mesoscale spatial heterogeneity of DFAA in the epilimnion of a lake. It is intriguing to speculate whether this observation indicates the presence of mesoscale patches with largely different DFAA concentrations. The spatial distribution of chlorophyll concentrations as well as bacterial abundances and activity significantly differ at both micro- and mesoscale (e.g., Seymour et al. 2004; Seymour et al. 2008) that may in turn contribute to the spatially uneven supply and consumption of DFAA. The observed spatial heterogeneity in DFAA concentrations might thus be indicative of habitat diversification characterized by areas with temporarily increased or decreased availability of DFAA. Nevertheless, the actual extent of spatial variations in DFAA needs to be further examined. For instance, the spatial heterogeneity of DFAA is expected to vary across depth or season.

In conclusion, we illustrate the complexity of processes governing the consumption and release patterns of glucose and amino acids during the spring period in the epilimnion of a lake. For one, synchronicity in the fluxes of leucine and glucose indicated that the microbial assemblages as a whole functioned like a generalist consumer. This was also supported by the similar behavior of individual DFAA during short-term dark incubations. Moreover, DFAA concentrations in these incubations tended to converge toward the seasonal median value of the in situ pool size, suggesting that this concentration represented a threshold for efficient microbial consumption. In this context, we noted that individual DFAA appeared to change more or less predictably, suggesting short-term differences in the coupling of release and consumption. Finally, we found that the temporal changes of total DFAA in the incubations were significantly related to their mesoscale spatial variability. This is a first indication that the heterogeneity of LMW DOM at the scale of meters may be directly affected by microbial processes.

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Conflict of Interest
None declared.