

**Amino acid and chlorin based degradation indicators in freshwater water systems**

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**Abstract**

Lakes cover a global area that is about 35 times smaller than the oceans, but carbon burial in lakes and oceans are on the same order of magnitude. Hence, understanding the processes for such high organic carbon burial in lacustrine systems is essential. We applied proxies typically used for marine environments including amino acid (AA) content and their nitrogen stable isotope composition to the water columns and sediments of three lakes that differ in their trophic states and deposition rates of sedimentary terrestrial organic matter. Additionally, we used carbon isotope fingerprinting of AAs to characterise their sources and fates. We show that this set of proxies tracks sources and degradation processes in eutrophic lakes with high sedimentary total organic carbon and nitrogen content. Those lakes also have a high total hydrolysable amino acid (THAA) content as well as higher pigment concentrations. While the Chlorin degradation Index (CI) showed increasing values with depth, the patterns were less consistent for the Degradation Index (DI). In general, all parameters failed to describe degradation in the most oligotrophic lake due to the very low organic carbon and nitrogen content in the sediment. We show that AAs are mostly of autochthonous origin and that AA

contribute 5-45 % to the organic material in plankton, POM, and sediment. Proxies based on AA for

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bacterial reworking ( $\Sigma V$ ) or trophic level (TL) showed increasing values in the water column but relatively stable values in the sediments. Furthermore, we show that methanotrophic bacteria led to increased values for the bacterial reworking proxy ( $\Sigma V$ ) and extraordinarily low  $\delta^{13}\text{C}$  AA values (-30 to -60 ‰).

## 1. Introduction

Only about 1.2 % of the Earth surface and 3 % of Earth's continental land surface is covered by lakes (Downing et al., 2006), an area much smaller than the area occupied by oceans (71 %). Owing in large part to transportation of terrestrial organic material (OM) into lakes, the global burial of organic carbon in lacustrine systems ( $0.04\text{--}0.09 \text{ Pg C yr}^{-1}$ ) is estimated to be up to three-quarters (Mendonça et al., 2017) of the burial occurring in the oceans ( $0.12 \text{ Pg C yr}^{-1}$ ) (Sarmiento and Sundquist, 1992). It is estimated that inland waters annually receive about  $1.9 \text{ Pg C yr}^{-1}$  from the terrestrial landscape (Cole et al., 2007) showing how important terrestrial organic carbon is.

The fate and reactivity of organic compounds depends on various parameters. In marine coastal sediments, for example, many variables including the oxygen content and productivity of the overlying water column, sediment accumulation rates (Stein, 1990), terrestrial input (Blair and Aller, 2012), and differences in the capacity of various minerals to sorb OM (Hedges and Keil, 1995; Keil et al., 1994) have been suggested as factors controlling the amount of organic matter preserved. Another factor triggering the preservation or degradation of organic material and hence its burial in sediments is the chemical composition of the organic material (Kharbush et al., 2020; Sun and Wakeham, 1994). While pigments are readily degraded in the water column, long-chain *n*-alkanes can persist in the sediment for millions of years.

The fate of OM in the water column and sediments has been studied extensively in marine environments, but these processes have received less attention in lacustrine environments. However, previous work has investigated amino acid-based degradation indices in the sediments of one lake (Meckler et al., 2004), compared lipid profiles in the water columns and sediments of two lakes with different trophic conditions (Bechtel and Schubert, 2009a, b), examined organic matter degradation in five lakes in relation to eutrophication and electron acceptors (Fiskal et al., 2019), investigated

specific lipids in sediments of a eutrophic lake (Naeher et al., 2012), and investigated sediments in Lake Geneva for terrestrial input from the Rhone river and its influence on electron acceptors and methane production (Randlett et al., 2015).

It is essential to characterize the molecular composition in the water column and sediments to understand the underlying processes of OM preservation in lakes. Several studies have concentrated on the amino fraction (amino acids, amino sugars) of the OM in oceans and lakes since those compounds are found in high concentrations in living organic material like phyto- and zooplankton as well as bacteria. For example, D/L enrichments in amino acids (AAs) showed that peptidoglycan remnants derived from bacterial cell walls constitute a major source of DON (McCarthy et al., 1998). Additionally, most degradation resistant dissolved organic nitrogen exists in the amide form (McCarthy et al., 1997). Nitrogen isotope compositions of AAs and associated proxies ( $\Delta Tr$ ,  $\Sigma V$ ) in oceanographic systems show a sharp divide between processing histories, and possibly sources, of particulate vs. dissolved AA (McCarthy et al., 2007). The trophic level indicator ( $\Delta Tr$ ) is derived from the difference between  $\delta^{15}N$  values of selected groups of AAs based on their relative enrichment with trophic transfer. The variance within a subgroup of AAs ( $\Sigma V$ ) indicates total AA resynthesis and is tied to heterotrophic microbial reworking in detrital materials (McCarthy et al., 2007). Work on high molecular weight dissolved organic nitrogen from phytoplankton cultures has further demonstrated the utility of these proxies (Calleja et al., 2013). Degradation of planktonic biomass into bacterial biomass can be tracked by characterizing amino sugars as demonstrated in a study off Peru (Niggemann and Schubert, 2006). This amino sugar approach, combined with uses of D-amino acids and the nitrogen isotopic composition of amino acids were tested in two lacustrine systems. The combined proxies showed higher bacterial degradation in an oligotrophic lake compared to a eutrophic lake (Carstens et al., 2012; Carstens et al., 2013; Carstens and Schubert, 2012).

Here we investigated the provenance and degradation of particulate OM in the water column and sedimentary OM in three different Swiss lakes: the eutrophic Lake Zug, the meso-eutrophic Lake Biel and the oligotrophic Lake Thun. We measured AA content and used the composition to calculate the degradation index (DI) (Dauwe and Middelburg, 1998; Dauwe et al., 1999). Compound specific stable nitrogen isotope composition of AA were used to calculate the bacterial reworking parameter  $\delta V$  (McCarthy, 2007 #19414) (McCarthy et al., 2007) and the trophic level (TLGlx/Phe, (Chikaraishi et al., 2009). Additionally, we calculated the chlorin index as a complementary degradation proxy as well as the chlorin concentration as an indicator for autotrophic production (Schubert et al., 2005). To reveal the terrestrial fraction of proteinogenic AAs, we used carbon stable isotope AA fingerprinting (Larsen et al., 2009; Larsen et al., 2012). Besides characterising the provenance and degradation of OM, we critically evaluated the proxies' applicability in our study system since many of those proxies were used extensively in marine but not lacustrine environments.

## 2. Experimental

### 2.1. Sample locations and collection

We examined four lake basins with different trophic states and different inputs of terrestrial matter to compare their nutrient states and allochthonous and autochthonous contributions to sediments. In Lake Zug, the northern basin (Lake Zug N) is partially anoxic and the southern basin (Lake Zug S) has a permanent anoxic bottom layer (Mengis et al., 1997). We sampled both basins to compare with two other lakes showing oxic water columns. The terrestrial contribution is low due to low river input and the eutrophic state of Lake Zug. Lake Biel, the second examined lake was the meso-eutrophic (Tschumi et al., 1982). We took samples near the inlet of the Aare River, which is the main tributary leading to a significant terrestrial contribution to the sediment. The set of lakes was completed with the highly oligotrophic Lake Thun where we expected a significantly higher terrestrial imprint compared to the other lakes due to low autotrophic production. A sediment core was taken near the inlet of the Kander River, responsible for 85 % of the allochthonous OM (Sturm and Matter, 1972). The average residence times of the water in the lakes are 14.1 years for Lake Zug, 2 years for Lake Thun and only 54 days for Lake Biel.

Sediment cores between 30 cm (Lake Zug) and 60 cm (Lake Thun) length were taken with a gravity corer (60 mm, without core catcher, Uwitec, Mondsee, Austria) that allowed for sampling of the sediment-water interface. Core locations were selected depending on the needed sediment (Lake Zug and Lake Biel more centrally for autochthonous material, Lake Thun in front of the river to sample more allochthonous material). Particulate organic matter from the water column was filtered on a GF/F filter (142 mm diameter, Schleicher & Schuell, combusted at 380°C) using an in-situ pump (McLane, East Falmouth, MA, USA). We measured basic water parameters (temperature, conductivity, and turbidity) using a CTD (Sea & Sun Technology, Trappenkamp, Germany) equipped with an oxygen electrode. The oxygen electrode was calibrated by Winkler titration with

water samples from different depths. We took plankton samples between the water surface and 30 m using a self-made plankton net with 35  $\mu\text{m}$  mesh size that was operated with a motor winch.

## 2.2. Elemental and isotopic composition

The organic carbon contents of sediment and GF/F samples were determined after carbonate removal using methods B and C, respectively, with slight modifications from Schubert and Nielsen (2000). Samples were acidified with 4 M HCl (Suprapure, Merck) and the open vials were subsequently heated at 50 °C for 1 h. Then samples were centrifuged and the supernatant was discarded. The residue was washed three times with MilliQ water and freeze-dried. The GF/F samples were stored in a desiccator containing a crystallising dish with 32 % HCl (Carl-Roth, Germany). The filters were removed after 72 hours and dried in an oven at 40 °C.

Elemental composition of total carbon (TC), total nitrogen (TN), and organic carbon ( $C_{\text{org}}$ ) were determined on dried and homogenised material weighed into tin capsules (Säntis Analytical, Switzerland). Sample  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were measured on an Elementar Vario PYRO CUBE CN elemental analyser (Elementar, Germany) connected to an isotope ratio mass spectrometer (IRMS, Isoprime, England). We added  $\text{V}_2\text{O}_5$  to the sediment sample (around 20 % of the sample mass) for complete combustion. Carbon and nitrogen isotope values were normalized to the Vienna Pee Dee Belemnite (VPDB) and atmospheric air scales, respectively, using mean measured values of an acetanilide standard for a given set of analyses, which was analysed after every tenth sample. The analytical precision of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were 0.2 ‰ and 0.4 ‰.

## 2.3. Chlorin Index

We calculated the chlorin index using the procedure of Schubert et al. (2005). In brief, chlorins were extracted from the sediment and the particulate material was filtered on GF/F filters with acetone and measured using a fluorimeter (Cary Eclipse, excitation wavelength 428 nm emission at 671 nm). To determine concentrations, chlorophyll *a* acidified with HCl (10 %) was used as a standard. Sample

extracts were acidified with HCl (10 %) to transform the chlorophylls to phaeophytin, which is an immediate diagenetic product of chlorophyll (Harris and Maxwell, 1995). The ratio of the fluorescence intensity before and after the acidification gives the chlorin index ( $CI = \text{Fluorescence Intensity}_{\text{acidified extract}} / \text{Fluorescence Intensity}_{\text{original extract}}$ ). The error of 10 single fluorescence measurements is better than 5 % resulting in a propagated error of 7 % for the CI. CI values are typically between 0.2 (fresh OM) and 1 for highly degraded OM (Schubert et al., 2005).

#### **2.4. Hydrolysis, derivatisation and quantification of amino acids**

Lipid-free sediment (extracted with MeOH/DCM 3:7 in a microwave, 7 min at 500 Watt) was hydrolysed with 6 M HCl at 110 °C for 20 h and L-Norleucine was added as an internal standard. An aliquot of the hydrolysed sample was purified using cation exchange chromatography (AG 50W-X8, 200-400 mesh size, Biorad) modified from Metges et al. (1996).

The AAs were derivatised to form n-acetyl i-propyl esters for gas chromatography (GC) analysis using the method of Corr et al. (2007). Additionally, an AA Standard mix (AAS18, Sigma-Aldrich) and an internal standard (L-Norleucine, Sigma-Aldrich) was derivatised to determine the response factors for each AA to the internal standard. In brief, the dried samples were isopropylated using 1 mL of acidified isopropanol (2.8M acetylchloride). The vials were flushed with argon, capped, vortexed and heated to 100°C for 1 h. The remaining isopropanol was removed at 40°C with a stream of nitrogen. 1 mL of the acetylation mixture (acetone :  $\text{NEt}_3$  : acetic anhydride; 5:2:1) was added to the dried samples, the vial was flushed with argon, capped, vortexed and heated to 60°C for 10 min. The remaining acetylation mixture was removed at room temperature with a gentle stream of nitrogen. Ethyl acetate (2 mL) and saturated sodium chloride solution (1mL) were added to the dry samples and mixed thoroughly. The organic phase was then transferred into a new vial and stored at -18°C until measurement.



An Agilent 6890 Series Gas-Chromatography System equipped with a Gerstel MultiPurposeSampler MPS2 and an InertCap 35 GC column (GL Sciences, 60 m x 0.32 mm x 0.50  $\mu$ m) was used to separate the AAs. A temperature program slightly modified from Wang et al. (2018) was used. The following AAs could be identified: alanine (Ala), asparagine/aspartic acid (Asx), glutamine/glutamic acid (Glx), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val). The reproducibility of AAs concentration was better than 1 %.

## 2.5. Compound specific stable isotope analysis of amino acids

The nitrogen isotopic composition of each AA was determined using an isotope ratio mass spectrometer (Delta V Advantage, Thermo) coupled via a combustion tube in which nitrogen compounds are first oxidized to NO<sub>x</sub> and then reduced to N<sub>2</sub> (1000°C) to a gas chromatograph (GC-IRMS, column and temperature program were the same as used for the quantification, section 2.4). Samples were measured in triplicate and an external standard (caffeine) with known  $\delta^{15}\text{N}$  value was measured before and after each three sample measurements and used to normalize the values to the atmospheric nitrogen isotope scale. Reproducibility was usually better than  $\pm 0.5$  ‰ but never exceeded 1 ‰.

The  $\delta^{15}\text{N}$  values for THAAs were calculated using the mol-% and  $\delta^{15}\text{N}$  value for each AA (equation 1).

$$\delta^{15}\text{N}_{\text{THAA}} = \sum(\delta^{15}\text{N}_{\text{AA}} \times \text{mol}\%_{\text{AA}}) \quad (1)$$

The  $\delta^{13}\text{C}$  values of the AAs were measured in the same way as the nitrogen isotope composition but only in duplicates due to a generally higher precision. A correction factor for the carbon isotope composition due to derivatisation was determined for each AA measuring the underivatized AAs on

an EA-IRMS (six replicates) and compare those to the derivatised AA measured by GC-IRMS. Standard deviations of the EA-IRMS and GC-IRMS measurements were between 0.04 and 0.08 ‰ and between 0.04 and 0.35 ‰, respectively. The correction factor (cf) was thereafter determined using the following equation (Corr et al., 2007):

$$cf_{AA} = \frac{\delta^{13}C_{NAIP} \cdot (\#C_{AA} + \#C_{added}) - (\#C_{AA} \cdot \delta^{13}C_{AA})}{\#C_{added}} \quad (2)$$

with  $\delta^{13}C_{NAIP}$  = carbon isotope composition of the derivatised AA  
 $\delta^{13}C_{AA}$  = carbon isotope composition of the pure AA  
 $\#C_{AA}$  = amount of carbon atoms in the pure AA  
 $\#C_{added}$  = amount of carbon atoms added during derivatisation

## 2.6. Degradation index

Dauwe and Middelburg introduced the DI based on the molar composition of the THAA providing information on the degradation state of organic material (Dauwe and Middelburg, 1998; Dauwe et al., 1999). The key of this proxy is that the mol-% of some AAs decreases upon degradation whereas this value increases for other AA (Dauwe and Middelburg, 1998). Glycine and threonine, which are constituents in cell walls, are preferentially preserved during sinking leading to an accumulation in the sediment. On the other hand, cell plasma AAs like tyrosine, phenylalanine and glutamic acid are depleted during sinking and further in the sediment (Dauwe et al., 1999). The DI is calculated as follows:

$$DI = \sum_i \left[ \frac{\text{var}_i - \text{AVG var}_i}{\text{STD var}_i} \right] \cdot \text{fac.coef.}_i \quad (3)$$

with  $\text{var}_i$  = original mole percentage of amino acid i  
 $\text{AVG var}_i$  = mean value of  $\text{var}_i$   
 $\text{STD var}_i$  = standard deviation of  $\text{var}_i$   
 $\text{fac.coef.}_i$  = factor coefficient for amino acid i

The lower the DI value is, the more degraded the AA derived OM is. We used the factor coefficients published by Dauwe et al. (1999) for the calculation of DI.

A proxy that describes bacterial degradation or reworking is  $\Sigma V$  (Calleja et al., 2013; McCarthy et al., 2007). This proxy is based on a scattering of the  $\delta^{15}\text{N}$  composition during bacterial reworking (sensu McCarthy et al. (2007) which leads to a higher “average deviation in the  $\delta^{15}\text{N}$  values of the trophic-AA (AA that are enriched in  $^{15}\text{N}$  with each trophic level) Ala, Asx, Glx, Ile, Leu, and Pro” (McCarthy et al., 2007).  $\Sigma V$  values  $<1$  are typical for fresh biomass whereas values  $>1.5$  indicate a high amount of bacterial heterotrophy.  $\Sigma V$  values between 1 and 1.5 can also originate from trophic transfers and can therefore not explicitly be attributed to bacterial reworking (McCarthy et al., 2007).  $\Sigma V$  values for all four lakes were calculated using equation 4 with a propagated standard analytical error using the reproducibility of the single AAs of around 0.3:

$$\Sigma V = \frac{1}{n} \sum |\chi_i| \quad (4)$$

with  $\chi_i = \delta^{15}\text{N}_i - \text{AVG}(\delta^{15}\text{N}_i)$  (= deviation)  
 $n$  = number of AA used for calculation  
 $\text{AVG}(\delta^{15}\text{N})$  = average of all  $\delta^{15}\text{N}$  values

## 2.7 Trophic level calculation

It is possible to estimate the trophic level (TL) of an organism in a food chain using the stable nitrogen isotope composition of specific AAs. The estimation is based on the increase of  $\delta^{15}\text{N}$  values of Tr-AAs with each trophic transfer whereas Src-AA retain their nitrogen isotope composition (Chikaraishi et al., 2009; McCarthy et al., 2007; McClelland and Montoya, 2002). Chikaraishi et al. (2009) showed that it is also possible to calculate the TL in an aquatic food chain by just using Glx as Tr-AA and Phe as Src-AA using the following equation with a propagated standard analytical error using the reproducibility of the single AAs of around 0.15:

$$\text{TL}_{\text{Glx/Phe}} = \frac{[\delta^{15}\text{N}_{\text{Glx}} - \delta^{15}\text{N}_{\text{Phe}} - 3.4]}{7.6} + 1 \quad (5)$$

For the geochemical applications presented here, interpreting CSIA-AA based TL data requires a rigorous estimation of uncertainty in values being compared. For further discussion of error calculation please see a detail discussion in Ohkouchi et al. (2017). Additionally, the nitrogen isotope composition of Glx and Phe at the base of the food web is significantly different between terrestrial and aquatic food chains (Chikaraishi et al., 2010). This is especially true if C<sub>3</sub>-plants, the dominant plant type in Europe and in the catchment areas of this study, build the basis of the food chain (Still et al., 2003). Therefore, equation 5 was slightly modified (Chikaraishi et al., 2010):

$$TL_{Glx/Phe} = \frac{[\delta^{15}N_{Glx} - \delta^{15}N_{Phe} + 8.4]}{7.6} + 1 \quad (6)$$

The propagated standard analytical error for TL using the reproducibility of the single AAs is the same as above and around 0.15.

## 2.8. Statistical methods

To characterise the nature of OM transformation, we compared  $\delta^{13}C$  patterns of the essential AAs (EAA) of the four basins to previously published data. This approach rests on the premise that broad taxonomic groups such as algae, bacteria, plants and vascular plants each have unique  $\delta^{13}C_{EAA}$  patterns. Thus, it is possible creating classification models from samples with known EAA origins. In this study, we used training data from Larsen et al. (2013) of vascular plants and laboratory cultured freshwater phytoplankton and bacteria. Since the two datasets came from two laboratories using different analytical methods, we first performed an inter-laboratory calibration determining the isotopic offsets for single AAs between our plankton samples and the phytoplankton of Larsen et al. (2013). The average offset across five EAA was  $-0.6 \pm 1.0$  ‰ (see Supplementary Table S1 for details). After correcting for the offsets between the two laboratories, we created a classification model based on the Larsen data by applying linear discriminate function analysis (LDA) using the MASS library in R version 3.4.3 (RCore, 2016). The  $\delta^{13}C_{AA}$  values were centred to the mean by first calculating the mean of the five EAAs and then subtracting the mean value from each individual AA.

### 3. Results

#### 3.1. Bulk properties

Both basins of Lake Zug showed the highest  $C_{org}$  and organic nitrogen (N) contents at the sediment surface ( $C_{org}$  up to 6.7 %), and decreasing values with increasing sediment depth (Fig. 1). Sediment between 3 and 5 cm depth in Lake Zug was influenced by slumping events due to the steep flanks of the southern basin (Moor et al., 1996). For both basins, N declined slightly faster with depth than  $C_{org}$  resulting in increased  $C_{org}/N$  ratios (Lake Zug N: 7.7 to 9.3; Lake Zug S: 7.2 to 14.8). Overall, the amount of THAA showed a similar pattern than  $C_{org}$  for both basins with decreasing content (Lake Zug N: from 227  $\mu\text{mol g}^{-1}$  to 24  $\mu\text{mol g}^{-1}$ ; Lake Zug S: 265  $\mu\text{mol g}^{-1}$  to 27  $\mu\text{mol g}^{-1}$ ) with increasing sediment depth (Fig. 2).

$C_{org}$  concentrations in Lake Biel were between 2 and 3 % with an exception at depths between 3 and 7 cm where increased  $C_{org}$  concentrations between 3.1 and 3.9 % were measured (Fig. 1). The N concentrations were also slightly higher resulting in higher  $C_{org}/N$  ratios for these depths (around 12). The higher  $C_{org}/N$  ratio indicative for terrestrial OM was supported by carbon isotope values of around -28 ‰ and could be linked to higher input from the nearby Aare delta. Below 9 cm sediment depth, the  $C_{org}/N$  were lower again around 8 and  $C_{org}$  and N values decreased to a minimum at 17 cm ( $C_{org}$  = 1.9 % and N = 0.2 %). THAA concentrations were usually below 50  $\mu\text{mol g}^{-1}$  and decreased from 9 cm (57.9  $\mu\text{mol g}^{-1}$ ) to 19 cm (37.1  $\mu\text{mol g}^{-1}$ ) and remained more or less constant below that depth (Fig. 2).

Lake Thun showed the lowest  $C_{org}$  (below 0.9 %) and N (below 0.1 %) values of all cores with decreasing concentrations in the upper 7 cm of the sediment and only minor changes below. THAA concentrations were more than 20 times lower than sediments in Lake Zug with decreasing concentrations in the upper 5 cm (from 8.5  $\mu\text{mol g}^{-1}$  to 5  $\mu\text{mol g}^{-1}$ , Fig. 2).

### 3.2. Composition of total hydrolysable amino acids (THAA)

The most abundant AAs in Lake Zug basins were alanine (Ala), glycine (Gly), aspartic acid (Asx) and glutamic acid (Glx) for the plankton, water column, and sediment (Fig. 3, Supplementary Information Table S2). The abundance of Glx was smaller in the water column than in plankton whereas the abundance of Asx and Gly was higher in the water column. The order of the four most abundant AAs in the water column was Ala > Asx > Glx > Gly for both basins. The abundance of Gly increased over the water column into the sediment where Gly was the most abundant AA (Lake Zug N: 19.4 mol-%, Lake Zug S: 19.0 mol-%). Ala, Asx and Glx were the other three most abundant AA in the sediment in this order. The abundance of glutamic acid (Glx) was lower in the sediment than in the water column and the plankton samples.

Lake Biel showed a similar pattern to Lake Zug for the four most abundant AAs in the plankton sample with the exception that Ala (10.6 mol-%) was slightly more abundant than Asx (10.5 mol-%, Fig. 3). However, Glx was the most abundant AA (13.4 mol-%) and Gly the fourth most (9.8 mol-%). Like for Lake Zug, the abundance of Gly increased over the water column into the sediment whereas Glx was depleted. Ala and Asx showed also an increased abundance in the water column but did not further increase in the sediment.

In Lake Thun, Glx (13.7 mol-%) and Asx (11.3 mol-%) were the two most abundant AA in the plankton sample followed by Gly (11.0 mol-%) and Ala (10.9 mol-%, Fig. 3). The abundance of Gly was very high with an increased abundance between 1.2 and 2.1 mol-% and increased even further over the water column into the sediment (16 mol-%). Unlike the other two lakes, Glx did not decrease in the water column where it was the second most abundant AA (13.9 mol-%). The most abundant AA in the water column was Asx (14.6 mol-%) higher than in the plankton sample.

### 3.3. Degradation indicators

The fraction of carbon built up by AAs decreased with increasing water depth and sediment depth for both basins in Lake Zug (Fig. 2). Particulate organic material (POM) at 10 m water depth consisted of 43 % THAA (Lake Zug N) and 37 % THAA (Lake Zug S), respectively and declined to 25 % in the top sediment layer and further with increasing sediment depth to a value of around 10 %. The degradation index (DI) showed a decrease from 1.5 in the plankton to below -0.5 in the sediment (Fig. 2). The chlorin index (CI) also indicated an ongoing degradation with depth in both the water column (0.2 to 0.4) and sediment (0.5 to 0.9) for both basins (Fig. 4 and Tab. 1). The concentration of chlorins decreased with increasing sediment depth and showed overall a similar pattern like the THAA concentration (Fig. 2 and 4). The sediment top layers had chlorin concentrations of 165 and 197  $\mu\text{g g}^{-1}$ , which declined with increasing sediment depth to values of 5 and 8  $\mu\text{g g}^{-1}$  for LZN and LZS, respectively.

The contribution of THAA to the POM pool at 10 m and 55 m was around 21 %. The THAA/  $C_{\text{org}}$  ratio varied between 5 % and 12 %. The DI decreased from 1.5 for plankton to 0.9 in the water column at 10 m and was further decreasing with increasing water depth to -0.2 near the sediment water interface. The DI near the sediment water interface was much lower in Lake Biel (-0.2) compared to Lake Zug (Lake Zug N: 0.6; Lake Zug S: 1.0) or Lake Thun (1.5). The CI also indicated degradation over the water column (Table 1) with an increase from 0.3 at 10 m to 0.6 at 55 m water depth and an increase with increasing sediment depth to 0.7 at 30 cm (Fig. 4). The average chlorin concentrations in Lake Biel ( $\bar{C} = 5.5 \mu\text{g g}^{-1}$ ) were much lower than in Lake Zug ( $\bar{C} = 80 \mu\text{g g}^{-1}$ ).

The AA fraction of POM in the water column of Lake Thun varied between 17 and 23 % at 10 and 135 m, similar to Lake Biel (Fig. 2). The THAA/ $C_{\text{org}}$  ratio decreased from the lowest water column sample into the sediment where the ratio in the top layer was only 6 % and was below 11 % in the whole sediment core. Unlike the other lakes, the DIs of the water column samples were all higher than for the plankton sample (DI = 1.3); at 10 m water depth, DI was highest (2.0) and decreased with increasing water depth to 1.8 at 85 m and 1.5 at 135 m. In the sediment, the DI increased from -

0.1 at 1 cm to 0.3 at 40 cm. The CI stayed constant in the water column with a CI of 0.30 at 10 m and 85 m and of 0.34 at 135 m. In the sediment, the CI decreased between 1 cm (CI = 0.40) and 7 cm (0.53) and remained constant below that depth. The chlorin concentrations ( $\bar{O} = 0.5 \mu\text{g g}^{-1}$ ) were on average 11 times lower than in Lake Biel ( $\bar{O} = 5.5 \mu\text{g g}^{-1}$ ) and 160 times lower than in Lake Zug ( $\bar{O} = 80 \mu\text{g g}^{-1}$ ).

### 3.4. Compound specific stable nitrogen isotope analysis

Proteinaceous AAs are grouped into trophic and source AA (McCarthy et al., 2007; Popp et al., 2007). Trophic AA (Tr-AA: Ala, Asx, Glx, Ile, Leu, Pro, Val) become  $^{15}\text{N}$ -enriched with each trophic transfer and are therefore usually the AAs with the highest  $\delta^{15}\text{N}$  values. On the other hand, source AA (Src-AA: Gly, Lys, Phe, Ser, Tyr) only show minor changes in the nitrogen isotope composition upon trophic transfers and have therefore generally low  $\delta^{15}\text{N}$  values. The  $\delta^{15}\text{N}$  value of Tyr and Lys could not be determined and were neglected for the calculation of the average  $\delta^{15}\text{N}$  value of Src-AA ( $\delta^{15}\text{N}_{\text{AA}}$  data in Supplementary information Table S3).

In Lake Zug N, the  $\delta^{15}\text{N}$  value of planktonic THAA was 8.3 ‰ whereas the Tr-AA had the highest  $\delta^{15}\text{N}$  values (mean:  $11.1 \pm 1.2$  ‰), followed by Src-AA ( $4.4 \pm 0.5$  ‰) (Fig. 5). This pattern changed at 10 m where THAA (6.8 ‰) as well as Tr-AA ( $8.3 \pm 1.7$  ‰) and Src-AA ( $3.8 \pm 2.7$  ‰) had lower  $\delta^{15}\text{N}$  values. Tr-AA ( $12.8 \pm 1.9$  ‰) and Src-AA ( $6.8 \pm 2.2$  ‰) as well as THAA (10.3 ‰) showed increased  $\delta^{15}\text{N}$  values at 60 m water depth together with a lower DI and THAA/ $C_{\text{org}}$  ratio for this depth. At 105 m, the  $\delta^{15}\text{N}$  value of THAA (3.0 ‰) was the lowest of the water column, plankton and sediment samples. The same was true for Tr-AA ( $4.1 \pm 2.7$  ‰) and Src-AA ( $0.8 \pm 2.4$  ‰). Whereas THAA ( $4.8 \pm 0.9$  ‰) and Tr-AA ( $7.5 \pm 1.4$  ‰) had markedly higher  $\delta^{15}\text{N}$  values in the sediment, Src-AA showed only a minor increase ( $0.9 \pm 1.1$  ‰).

The nitrogen isotope composition of planktonic THAA was slightly lower in Lake Zug S than in Lake Zug N (7.3 ‰) which was mainly due to the lower  $\delta^{15}\text{N}$  value of Tr-AA ( $9.5 \pm 1.8$  ‰). Src-AA



had a similar nitrogen isotope composition ( $4.2 \pm 1.2 \text{ ‰}$ ) than in the northern basin. Like Lake Zug N, Src-AA had also a slightly higher  $\delta^{15}\text{N}$  value ( $4.8 \pm 2.6 \text{ ‰}$ ) whereas Tr-AA had a lower nitrogen isotope composition ( $8.8 \pm 2.1 \text{ ‰}$ ). The nitrogen isotope composition of all AA groups decreased over the water column reaching a minimum near the sediment water interface (Tr-AA:  $0.4 \pm 2.2 \text{ ‰}$ ; Src-AA:  $-4.3 \pm 2.0 \text{ ‰}$ ; THAA:  $-1.0 \text{ ‰}$ ). The nitrogen isotope composition decreased over the sediment core for all AA groups. At 1 cm sediment depth, the  $\delta^{15}\text{N}$  value were  $11.0 \pm 3.0 \text{ ‰}$  for Tr-AA,  $5.4 \pm 2.0 \text{ ‰}$  for Src-AA, and  $8.0 \text{ ‰}$  for THAA reached a minimum at 30 cm sediment depth (Tr-AA:  $3.5 \pm 2.7 \text{ ‰}$ ; Src-AA:  $-0.2 \pm 2.1 \text{ ‰}$ ; THAA:  $2.0 \text{ ‰}$ ).

Lake Biel had the highest  $\delta^{15}\text{N}$  value of THAA in the plankton samples among the examined lakes ( $10.6 \text{ ‰}$ ). Both, Tr-AA and Src-AA, were relatively  $^{15}\text{N}$ -rich with  $\delta^{15}\text{N}$  values of  $13.9 \pm 1.9 \text{ ‰}$  and  $6.5 \pm 0.6 \text{ ‰}$ , respectively. Furthermore, the AA at 55 m water depth were extremely  $^{15}\text{N}$ -rich representing the highest  $\delta^{15}\text{N}$  values of the whole dataset of this publication (Tr-AA:  $21.0 \pm 1.5 \text{ ‰}$ ; Src-AA:  $13.6 \pm 4.0 \text{ ‰}$ ; THAA:  $18.0 \text{ ‰}$ ). On the other hand, the  $\delta^{15}\text{N}$  values at 10 m water depth were similar to Lake Zug (Tr-AA:  $9.2 \pm 1.9 \text{ ‰}$ ; Src-AA:  $4.1 \pm 1.0 \text{ ‰}$ ; THAA:  $7.4 \text{ ‰}$ ). Furthermore, the nitrogen isotope composition of the sediment was also similar to Lake Zug N (Tr-AA:  $7.0 \pm 1.1 \text{ ‰}$ ; THAA:  $5.1 \pm 1.1 \text{ ‰}$ ) except Src-AA which were less  $^{15}\text{N}$ -depleted ( $3.1 \pm 1.0 \text{ ‰}$ ) compared to Lake Zug N.

Lake Thun generally showed the lowest  $\delta^{15}\text{N}$  values among our dataset. The  $\delta^{15}\text{N}$  value of the plankton sample was  $3.6 \text{ ‰}$  which was mainly due to very low  $\delta^{15}\text{N}$  values for Src-AA ( $-0.9 \pm 0.9 \text{ ‰}$ ). The nitrogen isotope composition of Tr-AA was  $6.5 \pm 1.5 \text{ ‰}$ . At 10 m water depth, the AAs were even more  $^{15}\text{N}$ -depleted (THAA:  $-1.4 \text{ ‰}$ ) which was due to the  $\delta^{15}\text{N}$  values Src-AA ( $-5.9 \pm 0.9 \text{ ‰}$ ) and Tr-AA ( $-0.2 \pm 1.9 \text{ ‰}$ ) which were yet lower than in the plankton. Tr-AA did also show increasing  $\delta^{15}\text{N}$  values over the water column (85 m:  $5.3 \pm 2.5 \text{ ‰}$ , 135 m:  $8.7 \pm 1.7 \text{ ‰}$ ) as well as THAA (85 m:  $3.8 \text{ ‰}$ , 135 m:  $4.9 \text{ ‰}$ ). The Src-AA at 85 m water depth were also  $^{15}\text{N}$ -enriched ( $0.3 \pm$

2.9 ‰) compared to the 10 m sample but remained constant below. The sediment was  $^{15}\text{N}$ -depleted compared to the 135 m POC sample with an average  $\delta^{15}\text{N}$  value of  $0.2 \pm 0.7$  ‰ for THAA.

### 3.5. Compound specific stable carbon isotope analysis

We examined 11 AAs of which five are considered essential for animals (EAA: Ile, Leu, Phe, Thr, Val) and six are considered non-essential (NEAA: Ala, Asx, Gly, Glx, Pro, Ser;  $\delta^{13}\text{C}_{\text{AA}}$  data in Supplementary Information Table S4). This distinction is relevant for our study because the EAA are more informative of sources than the NEAA as per Larsen et al. (2009, 2013). Among the four basins, the two Lake Zug basins had the most variable and  $^{13}\text{C}$  depleted POC values (Fig. 7).

For Lake Zug North,  $\delta^{13}\text{C}_{\text{AA}}$  values of POM collected at 105 m depth were 15-20 ‰ more negative compared to surface plankton (Fig. 7, Table S4). For Lake Zug South, POM was more  $^{13}\text{C}$  depleted at 155 m than at 185 m and at 155 m  $\delta^{13}\text{C}_{\text{AA}}$  values were 10-20 ‰ more negative compared to plankton (Fig. 7). In comparison, Lake Thun's two deepest POM samples at 85 m and 135 m were generally less than 10 ‰ depleted compared to plankton. In Lake Biel, the shallowest basin, POM from 55 m generally resembled plankton except for Val that was  $^{13}\text{C}$  depleted by 11 ‰ compared to plankton. The  $\delta^{13}\text{C}_{\text{AA}}$  values of the sediment samples resembled the  $\delta^{13}\text{C}_{\text{AA}}$  values of the plankton samples more closely than those of the POM samples (Fig. 7). Except for the Lake Thun sediment samples,  $\delta^{13}\text{C}_{\text{AA}}$  patterns were consistent across sediment depth despite shifts in  $\delta^{13}\text{C}$  baseline values. In Lake Thun, Val and Phe exhibited contrasting  $\delta^{13}\text{C}$  trends with depth (Fig. 7).

To predict the EAA origins in the plankton, POM and sediment samples, we created a classification model with training data from Larsen et al. (2013) comprising of vascular plants and laboratory cultured freshwater phytoplankton and bacteria (Fig. 8). To test the robustness of the LDA classification, we performed the leave-one-out cross validation that showed all training data samples classified correctly (>99.99 % confidence, LDA coefficients and traces in Supplementary Information Table S1). In the following, we denote source diagnostic  $\delta^{13}\text{C}_{\text{EAA}}$  patterns as fingerprints

as per Larsen et al. (2009). According to the LDA, the  $\delta^{13}\text{C}_{\text{EAA}}$  fingerprints of plankton samples from Lake Biel and Lake Thun resembled algae (i.e. laboratory grown phytoplankton) more closely than plankton from Lake Zug (Fig. 8A). POM generally classified as algae except for POM collected at >100 m depth, which classified as plants (Fig. 8B). All sediment samples from Lake Biel, Lake Zug, and the Lake Thun surface sediment sample (1 cm) resembled algae, whereas the other sediment samples from Lake Thun resembled bacteria (Fig. 8C).

## 4. Discussion

Our study applied and tested the combination of a number of AA- and chlorophyll-based proxies mainly used in marine studies, with the aims of 1) characterizing the origins, transformation, and fate of OM in four lake basins with different trophic status and different input of terrestrial matter and 2) to critically assess the proxies' applicability in lacustrine systems.

### 4.1. Degradation indicators

Looking at the CI, Lake Zug and Lake Biel behave like textbook examples with CI values of 0.2 for plankton and 0.9 for strongly degraded OM in the sediments. In addition, high correlations between  $C_{\text{org}}$  and chlorin concentrations of  $r^2 = 0.92$  for Lake Zug N and of  $r^2 = 0.94$  for Lake Zug S and an  $r^2 = 0.48$  for Lake Biel indicate that both parameters show a similar degree of degradation. In the oligotrophic lake Thun, the concentration of chlorins were only one-thirtieth (0.1‰ of  $C_{\text{org}}$ ) compared to eutrophic Lake Zug. With this low amount it is questionable whether an index like the chlorin index (CI) can be used to describe the overall OM degradation state of sediments or POM. The overall autochthonous OM contribution to Lake Thun is very low with five times smaller  $C_{\text{org}}$ , N values, and 25 times smaller THAA concentrations in the surface sediments than sediments in Lake Zug. Furthermore, the lignin content indicative for allochthonous OM in Lake Thun is three times higher than that of Lake Zug N (data not shown). Hence it is difficult to characterise a lake with

sparse autochthonous OM with parameters that target on chlorophyll or amino compounds derived from phyto- and zooplankton.

In a second step, we concentrated on the bulk AAs (THAA) and the DI which is used as a degradation index (Dauwe et al., 1999). Correlating THAA against  $C_{org}$  reveals an  $r^2 = 0.96$  and  $r^2 = 0.93$  for the northern and southern basins of Lake Zug, respectively. In Lake Biel, the corresponding correlation between THAA and  $C_{org}$  is lower at  $r^2 = 0.65$ . These results show that the amount of THAA is highly correlated to the  $C_{org}$  content supporting that THAA is a large fraction of the OM with decreasing contents from the uppermost water column with fresh plankton towards the deeper sediments. This is furthermore recorded by the decreasing DI values that showed values between 1.6 and 0.8 in the water column and then a smoother decrease in the sediments of all three lakes down to  $DI = -0.8$ . When comparing POM degradation in the water column of Lake Zug and Lake Biel, it is obvious that the degradation in the water column of Lake Biel is stronger with a lower DI and a higher CI in the deepest sample. As previously shown in the highly oligotrophic Lake Brienz, POM is strongly degraded in the water column (Carstens and Schubert, 2012), which the authors attributed to the fact that small particles have a longer residence time in the water column than large particles and hence are prone to greater degradation. However, we can only speculate whether the same mechanism worked in Lake Biel since we have no data on particle sizes in the water column. In Lake Zug S, all three degradation indicators depicted degradation until 155 m near the oxycline. Below 155 m, DI and the THAA/ $C_{org}$  ratio remained constant, only CI slightly increased. The reason might be the onset of anoxic conditions below 155 m which typically lead to slower degradation (Gelinas et al., 2001; Jessen et al., 2017; Sobek et al., 2009).

In conclusion, both indicators, CI and DI, are suitable to describe the degradation first in the water column and later in the sediments. It was noted earlier that the CI seems to be advantageous in describing degradation in an earlier stage whereas the DI might describe degradation better in a later

stage (Meckler et al., 2004). In our study, the CI and DI depicted an ongoing degradation in the sediment of Lake Zug N, S, and Lake Biel until a sediment depth between 20 to 30 cm where the CI reached maxima. However, the DI indicates an ongoing degradation until a sediment depth of 40 cm in Lake Zug N, whereas in the other two lakes the DI stays rather constant with a higher scatter. Hence, in this study it is unclear whether one or the other proxy would be more appropriate in describing degradation states.

In the water column of Lake Thun, the CI indicated little degradation, whereas DI and THAA/C<sub>org</sub> implied ongoing degradation in the water column. Nevertheless, degradation in the water column of Lake Thun (DI = 1.3 for plankton and 1.5 at 135 m) seemed less than in the other lakes. The DI in the sediment (-0.1) is then much lower suggesting that most degradation occurred at the sediment water interface. As stated before, neither DI nor the THAA/C<sub>org</sub> ratio seem to be suitable to describe the degradation state in the sediment of Lake Thun. The DI even showed an increasing trend with increasing sediment depth that would lead to the conclusion that the OM is getting fresher with increasing sediment depth, which seems at first sight irrational but could well be if degraded OM is transformed via heterotrophy to new bacterial biomass production.

#### 4.2. Amino acids as source markers

The Gly/Ser ratio might be useful to estimate the relative abundances of diatoms to bacteria because Gly is more abundant in the bacterial cell wall polymer peptidoglycan relative to that in the frustules of diatoms, and vice versa for Ser (Hecky et al., 1973). Therefore, this ratio has been used to estimate the origin of the autotrophic production (Ingalls et al., 2003; Niggemann et al., 2018). The low Gly/Ser ratios in Lake Zug (Fig. 6) for the samples at 10 m water depth (Lake Zug N: 1.3, Lake Zug S: 1.1) and plankton (Lake Zug N: 1.2, Lake Zug S: 1.3) are similar to Gly/Ser ratios found in diatoms (Cowie et al., 1992; Hecky et al., 1973; Ingalls et al., 2003) indicative of high diatom production (Amt für Umweltschutz Zug, 2004). The ratio increased over the water column to 1.5 for

both basins indicated an increased contribution of peptidoglycan compared to diatom frustules and hence bacterial degradation (Ingalls et al., 2003; Niggemann et al., 2018).

Lake Biel, in which diatoms only account for about 20 % of the phytoplankton biomass (AWA Kanton Bern, Gewässer- und Bodenschutzlabor), showed a higher Gly/Ser ratio at 10 m water depth (1.5). Increasing Gly/Ser ratio indicate bacterial reworking of diatom frustules in the sediment (see Lake Zug).

In Lake Thun the Gly/Ser ratio in plankton (1.5) was similar to Lake Biel. Furthermore, the Gly/Ser ratio at 10 m in Lake Thun was very high compared to the other lakes (2.6). Although diatoms are responsible for around one-third of the total phytoplankton biomass (AWA Kanton Bern, Gewässer- und Bodenschutzlabor) it seems that the diatom frustules are reworked rapidly and transformed into bacterial biomass. This tight coupling of autotrophic and heterotrophic processes in oligotrophic systems has been described before (Carstens et al., 2013; Cotner and Biddanda, 2002).

#### **4.3. Bacterial reworking**

Increasing mol-% of Gly are generally attributed to an increasing microbial activity (Dauwe and Middelburg, 1998; McCarthy et al., 2007) since Gly, as well as Ala and Thr are enriched in the cell wall polymer peptidoglycan. This cell wall AA (CW-AA) composition is unique for bacteria (Hecky et al., 1973; Schleifer and Kandler, 1972). On the other hand, the abundance of the AAs Tyr, Phe and Glx is higher in the cytoplasm, hence called CP-AA (Hecky et al., 1973; Schleifer and Kandler, 1972) which is not restricted to bacteria but found in a wide variety of organisms (Sicko-Goad and Ladewski, 1977). Therefore, the ratio of CP-AA to CW-AA is indicative of the abundance of bacteria relative to other organisms, e.g. plankton. The plankton samples of all four basins, in which the bacterial contribution is expected to be low due to the mesh size of the plankton net (35µm), showed a similar CW-AA/CP-AA ratio between 1.3 and 1.4 (Tab. 2). The POM deeper in the water column increased to values between 1.9 and 2.3 in Lake Zug and Lake Biel. On the other hand, Lake

Thun retained its low value through all water depths showing no further transformation of plankton to bacterial biomass. The sediment values of all lakes showed higher values compared to the water column values (2.3 to 2.7) supporting ongoing degradation.

Increasing mol-% CW-AA might also originate from the degradation of microbial biomass leading to an accumulation of the more refractory cell wall polymer peptidoglycan (Keil et al., 2000; Müller et al., 1986; Siezen and Mague, 1978). It is hence not possible to distinguish between increased living bacterial biomass and a higher amount of degraded bacterial matter. However, CP-AAs degrade relatively fast (Dauwe et al., 1999), and therefore degradation always leads to increasing CW-AA/CP-AA ratios irrespective of being living or dead bacterial biomass.

The OM in the water column of Lake Thun seems very fresh based on the applied CW-AA/CP-AA ratios; however, as mentioned before,  $C_{org}$  as well as the AA content in the sediments are very low and the capabilities of those indicators are questionable. Nonetheless, for Lake Zug S and Lake Biel, the increasing CW-AA/CP-AA ratio seems to describe degradation satisfactorily.

The plankton sample of Lake Zug N had a low  $\Sigma V$  value (0.7) representative for fresh biomass (Fig. 6). At 10 m water depth the  $\Sigma V$  value had already doubled (1.4) indicating either bacterial reworking or an increase in trophic level. Since the TL value at 10 m water depth is around 1 (see section 4.4) the increased  $\Sigma V$  values at 10 m is the result of bacterial reworking which is also supported by the slightly increased CW-AA/CP-AA ratio at 10 m (see above). At 65 m water depth, the  $\Sigma V$  value is similar to the one at 10 m (1.5) whereas it increased at 105 m to 2.4. This high value near the sediment water interface indicates high bacterial activity or high abundance of reworked particles at this depth. Additionally, a low carbon isotope value of -47.6 ‰ for THAA at this depth hints towards methane oxidizing bacteria, which are known to be a substantial part of the microbial community in Lake Zug (Oswald et al., 2016). No clear pattern for the  $\Sigma V$  value was observed for the sediments in Lake Zug N (Fig. 6) besides a high Gly/Ser ratio (3.1 – 3.5) together with a high  $\Sigma V$  value at 15 cm (3.0) between 13 and 19 cm sediment depth suggesting increased bacterial reworking.

In the southern basin, the  $\Sigma V$  values are slightly higher compared to the northern basin (1.2). This might be due to the different sampling times of the two basins. The southern basin was sampled in the morning and the northern basin in the afternoon when vertical zooplankton migration (e.g. (Hutchinson, 1967; Lampert, 1989)) might be responsible for increased  $\Sigma V$  values in the plankton sample (Fig. 6). Similar to the northern basin, a  $\Sigma V$  value of 1.9 at 10 m water depth indicates a pronounced bacterial reworking in the photic zone of the southern basin. The bacterial reworking is even more distinct near the oxycline at 155 m with a  $\Sigma V$  of 2.4 which is also supported by an increased CW-AA/CP-AA ratio. In the sediment, the  $\Sigma V$  values remained constant (between 2.4 and 2.9) indicating high bacterial reworking.

No real trend in  $\Sigma V$  values could be found in the sediment of Lake Biel (Fig. 6). However, Lake Biel already showed very high  $\Sigma V$  values for plankton (1.6) and at 10 m water depth (1.8). Therefore, bacterial reworking near the water surface is high. At 55 m water depth the DI and CI suggest high bacterial reworking (Fig. 2, 4) and a high CW-AA/CP-AA ratio suggests a microbial contribution despite low  $\Sigma V$  values. Remarkable were the increased  $\delta^{15}\text{N}$  values for all AA (Tr, Src, THAA) at 55 m water depth. This increase cannot be explained by a trophic transfer because it would mainly affect the nitrogen isotope composition of Tr-AA whereas Src-AA would be unaffected. However, the  $\delta^{15}\text{N}$  values of the Src-AAs also increased. A similar behaviour of the nitrogen isotopic composition of AA has previously been attributed to isotopic fractionation associated with extracellular hydrolysis of protein to oligomers (Hannides et al., 2013) by microbes in natural environments to degrade peptides into smaller molecule fragments that can thereafter be assimilated (Hoppe et al., 2002). During hydrolysis, the preferential cleavage of  $^{14}\text{N}$ -C peptide bonds generally leads to higher  $\delta^{15}\text{N}$  values of the remaining AAs in the residual peptide (Bada et al., 1989; Silfer et al., 1992), which would explain the high  $\delta^{15}\text{N}$  values at 55 m water depth of Lake Biel. However, the hypothesis that extracellular hydrolysis causes such a pattern in the nitrogen isotope composition



relies on the assumption that the nitrogen isotope fractionation is similar for all peptide bonds and therefore needs further investigation (Ohkouchi et al., 2017).

Similar to Lake Zug, low  $\Sigma V$  values of the plankton samples indicated fresh OM in Lake Thun (Fig. 6). In contrast to the CW-AA/CP-AA ratio, increasing  $\Sigma V$  values with increasing water depth indicated bacterial reworking in the water column. The low abundance of CW-AAs could be explained by a dominance of gram-negative bacteria in Lake Thun, which have a substantially different molecular structure of the cell wall with a low fraction of peptidoglycan (Schleifer and Kandler, 1972). Similar to the other lakes, no clear pattern for the  $\Sigma V$  values in the sediments could be observed (Fig. 6).

#### 4.4. Trophic level

It is possible to estimate the trophic level (TL) of an organism in a food chain using the stable nitrogen isotope composition of specific amino acids (see equation 5 in methods). The nitrogen isotope composition of Glx and Phe at the base of the food web can be significantly different between terrestrial and aquatic food chains (Chikaraishi et al., 2010). Therefore, a mixing model was applied to calculate the TL in an aquatic system with high terrestrial influence such as rivers or lakes (Ishikawa et al., 2014; Ohkouchi et al., 2017). Unconventionally, our samples comprised of mixed living and dead materials such as plankton, POM, and sediment samples rather than living organisms such as phyto-, zooplankton, small fish, big fish, etc. (Ishikawa et al., 2014; Ohkouchi et al., 2017). However, we wanted to get an idea of the TL of material integrating over all sedimenting OM including bacteria that also elevate TL (Steffan and Dharampal, 2019).

We estimated the terrestrial contribution using a simple mixing model based on the carbon isotope composition of the OM in the sediment applying a terrestrial and lacustrine endmember (Stücheli, 2018). Using a phytoplankton  $\delta^{13}\text{C}$  value between -32.7 (Lake Thun) and -38.6 ‰ (Lake Zug) and a terrestrial endmember of -26 ‰, the terrestrial contribution was estimated to be between 93 and 28

%, respectively. Taking the terrestrial contribution into account (using equation 6) led to TL in the sediment between 1.5 and 2.3, which is a higher than could be expected for zooplankton (TL=2, Fig. 6). In contrast, the estimation of the TL not considering the terrestrial contribution (using equation 5) led to TL between 0.5 and 1.5 (being too low, i.e. between phytoplankton corresponding to TL = 1 and zooplankton).

In Lake Zug N, the trophic level of the plankton sample (TL = 1.6) indicated a substantial contribution of zooplankton, whereas at 10 m almost only phytoplankton, (TL = 0.9) was found. Thus, zooplankton might be grazing on living phytoplankton near the surface, whereas deeper in the water column dead biomass is preferably consumed (TL at 60 m = 1.6). In the sediment, the TL showed an average value of  $2.2 \pm 0.1$  and did not change much with increasing sediment depth.

The southern basin of Lake Zug showed a similar pattern for TL like the northern basin with a plankton sample value of 1.4 a TL = 1.0 at 10 m water depth TL = 1.1 and 1.3 at 155 m near the oxycline and at 185 m close to the sediment whereas the latter could be related to resuspension of OM from the sediment or dead zooplankton sinking towards the sediment. Another possible explanation would be reworking by bacteria causing the TL value to increase (Steffan et al., 2015; Yamaguchi, 2012; Yamaguchi et al., 2017). The TL remains constant in the sediment below 5 cm ( $2.3 \pm 0.1$ ).

The plankton sample in Lake Biel had a TL of 1.9, which was the highest value for all water column/plankton samples among all lakes indicating a strong contribution of zooplankton to the plankton community. The trophic level at 10 m and 55 m (TL = 1.4 and 1.3) was lower than the trophic level of the plankton sample but still indicated an influence of zooplankton. In the sediment, TL showed an increase with sediment depth below 9 cm (TL = 1.7 to 2.1) probably indicating grazing in the oxic sediment (e.g. by oligochaetes).

The trophic level of plankton in Lake Thun ( $TL = 1.7$ ) indicated a substantial contribution of zooplankton to the plankton community. However, in contrast, to the bacterial reworking, the trophic level decreased with increasing water column (10 m:  $TL = 1.5$ , 85 m:  $TL = 1.3$ ) and increased again near the sediment water interface ( $TL_{Glx/Phc} = 1.6$ ) maybe related to resuspension of OM. For the sediment, the trophic level varies around  $2.0 \pm 0.2$  as an integration of all sedimented OM, similar to the other lakes.

#### 4.5. Carbon isotopes of amino acids

Amino acid  $\delta^{13}C$  fingerprinting is an emerging method for understanding the origins and fate of OM in aquatic systems (Larsen et al., 2015; McMahon et al., 2015). As expected, plankton  $\delta^{13}C_{EAA}$  fingerprints from all four basins resembled those of algae, i.e. laboratory cultured phytoplankton; however, the fingerprints from Lake Zug differ from those of Lake Biel and Lake Thun indicating that their algal assemblages are different (Fig. 8). The POM samples fell along a transect between algae and terrestrial plants. Surprisingly, Lake Zug samples below 100 m clustered with the terrestrial plants. It is highly unlikely that plants were the source of these EAA given that their  $\delta^{13}C$  values were 10-20‰ more negative than EAA of surface plankton. Rather, the combination of very negative  $\delta^{13}C$  values and vague resemblance to algal fingerprints indicates a non-autotrophic EAA source such as methanotrophs. The southern basin of Lake Zug has a chemocline where methane-oxidizing bacteria are an important part of the microbial community (Oswald et al., 2017; Oswald et al., 2016). If methanotrophs were indeed the source of these EAA, then our results indicate that methanogenic bacteria in these Swiss lakes have  $\delta^{13}C_{EAA}$  fingerprints that resemble vascular plants more than the bacteria cultured by Larsen et al. (2013). Sediment samples resembled algae except for samples from Lake Thun that resembled bacteria, supporting the interpretation that bacterial reworking was greatest in Lake Thun. For the two basins in Lake Zug, each sediment layer had almost comparable linear discriminant scores indicating similar primary production sources and

degradation processes in the two basins. For Lake Biel, sediment discriminant scores across all layers were almost indistinguishable despite variations in  $\delta^{13}\text{C}$  baseline values. A likely explanation is that the rate of microbial reworking is very slow, and that primary production have remained consistent during the past 30 years.

The potential of using  $\delta^{13}\text{C}_{\text{EAA}}$  fingerprints to identify microbial EAA origins and modes of carbon acquisition was first demonstrated by Scott et al. (2007). Our findings show that the current fingerprinting training data are inadequate for characterizing biogeochemical processes in deeper water layers, and that it will be key for future studies obtaining training data for methanotrophic stains such as *Crenothrix* (Oswald et al., 2017), gammaproteobacterial methanotrophs (Oswald et al., 2016), and locally relevant phytoplankton taxa such as freshwater diatoms and golden algae.

#### 4.6. Implications

It is important to understand OM degradation and preservation to be able to estimate the OM that is buried in the sediments of oceans and lakes. Here we applied several indicators that describe the freshness of OM and the contribution of bacterial reworking and biomass to sinking and deposited OM in lakes of different trophic status. Whereas those proxies were able to fulfil their purpose in the eutrophic and mesotrophic lakes they failed to work in the oligotrophic lake. The OM material produced in the oligotrophic lake and subsequently deposited in the sediment was too little for the proxies to work. In the other lakes the high contribution of the AA to the OM showed similar degradation dynamics and were successful in describing overall degradation in the water column and sediments. The ratio of cell wall to cell plasma AA did not work in the sediments. Although chlorins were only a small fraction of the OM they had similar degradation dynamics than the overall OM which made them a useful and fast to apply proxy. The usefulness of the nitrogen isotope composition of single AA and both related proxies need further investigations. Whereas they gave some hints about water column processes they were indistinct in the sediments; a fact that might

have been expected due to the countless components of sedimentary OM. Similarly, the  $\delta^{13}\text{C}_{\text{EAA}}$  fingerprints need training data for the investigated environment before it could be used successfully. Overall, it has to be stated that the one perfect proxy to describe OM degradation in water column and/or sediments does not exist but the melange of constituents in OM makes it necessary to rely on numerous proxies.

## 5. Conclusions

The studied degradation proxies showed that bacterial reworking is high in the oligotrophic Lake Thun, which might be due to tight coupling of heterotrophic and autotrophic processes in oligotrophic systems as described before. Eutrophic Lake Zug showed high bacterial reworking at water depths with low oxygen concentrations. Here, the carbon isotope composition of THAA was very low with  $\delta^{13}\text{C}_{\text{AA}}$  values between -30 and -60 ‰. These low  $\delta^{13}\text{C}$  values were linked to methane oxidizing bacteria previously found at that depth (Oswald et al., 2016). For Lake Biel, the common proxy for bacterial reworking  $\Sigma\text{V}$  did not indicate major bacterial reworking. Additionally,  $\delta^{13}\text{C}_{\text{EAA}}$  fingerprints also suggest minor bacterial reworking in Lake Biel. In contrast to that, the ratio of cell wall AAs and cell plasma AAs (CW-AA/CP-AA ratio) was high suggesting high bacterial biomass near the sediment water interface. Additionally, the proxies for degradation (DI and CI) and very high  $\delta^{15}\text{N}$  values around 20 ‰ near the sediment water interface indicated a pronounced degradation in Lake Biel. This discrepancy was explained by a recently discovered process called extracellular protein hydrolysis (Hannides et al., 2013). This microbial process leads to a similar isotope fractionation for all AAs and neither  $\Sigma\text{V}$  nor  $\text{TL}_{\text{Glx/Phe}}$  increase during that process. However, this process is still a hypothesis and needs further investigation.

Furthermore, we showed that more realistic values for the TL in lacustrine systems can be obtained by taking the terrestrial OM contribution to the sediment into account. Neither  $\Sigma\text{V}$  nor  $\text{TL}_{\text{Glx/Phe}}$

showed any trends in the sediment which makes the use of those two proxies for sediment samples questionable.

Finally, AA  $\delta^{13}\text{C}$  fingerprinting was used to examine the origin of the AAs. This method verified that the AAs are mostly of autochthonous origin. Additionally, a strong bacterial reworking in Lake Thun could be revealed due to a high resemblance of the sediment with bacterial training data. However, our results also showed that the current training data for fingerprinting AA sources in lacustrine systems are insufficient. The POM samples of Lake Zug were classified to originate in vascular plants. However, the eutrophic state suggested a high autotrophic influence rather than a high terrestrial influence. Therefore, the training data is limited and is not applicable for every lake habitat. To address this issue, it would be essential for future studies to obtain training data for the most relevant taxa for a given study site; in our case methanotrophic strains such as *Crenothrix*, gammaproteobacteria related to methanotrophy, and the most dominant phytoplankton taxa in the four basins such as diatoms and golden algae.

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**Table 1.** CW-AA/CP-AA ratio for the four lake basins

**Figure 1.** Concentrations of  $\text{C}_{\text{org}}$ , N, and  $\text{C}_{\text{org}}/\text{N}$  ratios of all four basins: Lake Zug northern basin LZN, Lake Zug southern basin LZS, Lake Biel LB, Lake Thun LT.

**Figure 2.** Total hydrolysable amino acids (THAA in  $\mu\text{g/g}$  dry weight sediment), THAA/ $\text{C}_{\text{org}}$  ratio (in %) and the amino acid based degradation index (DI) of all four basins. Note different axis for Lake Zug, Lake Biel, and Lake Thun.

**Figure 3.** Concentration of amino acids of total hydrolysable amino acids (THAA) in mol-% of plankton, water column and sediment samples for all four basins. Because only one plankton sample was measured for each basin, no standard deviation could be determined. For the same reason, no standard deviation of the water column for Lake Biel could be calculated.

**Figure 4.** Chlorin Index and chlorin concentrations of all four basins and the chlorin **fraction** of  $C_{org}$  and the chlorin and THAA content.

**Figure 5.** Average stable nitrogen isotope composition of source (Src-AA) and trophic (Tr-AA) amino acids as well as the calculated nitrogen isotope composition for THAA for all four basins (a) LZN, (b) LZS, (c) LB, (d) LT.

**Figure 6.** Mol-% of Ser (triangles), CP-AA (Tyr, Phe and Glx; squares) and CW-AA (Gly, Thr and Ala; circles). The Gly/Ser ratio was calculated to determine the influence of diatoms and its bacterial reworking. The bacterial reworking ( $\Sigma V$ ) and the trophic level (TL) of several samples were determined using stable nitrogen isotopes. Errors for  $\Sigma V$  and TL are 0.3 and 0.15, respectively.

**Figure 7.** Comparison of amino acid  $\delta^{13}C$  values across basins and sample types. The symbols denote mean values and the bars standard deviation values.

**Figure 8.** Linear discriminant analysis (LDA) plot of  $\delta^{13}C_{EAA}$  (Ile, Leu, Phe, Thr, Val) values based on training data from Larsen et al. 2013. (A) Plankton from Lake Biel and Lake Thun resemble algae (short for laboratory grown phytoplankton) more than plankton from Lake Zug. (B) POC generally classify as algae except for samples collected at >100 m depth that classify as plants (numbers adjacent to the symbols signify depth). (C) All sediment samples for Lake Biel and Lake Zug resemble algae with the Lake Biel samples having the least variability. Sediment samples from Lake Thun resemble bacteria more than algae except for the surface sediment sample (1 cm) that classify as algae. The ellipses signify 95% confidence boundaries for each salmon group, and the broken lines signify decision boundaries for the classified samples.

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		DI	CI	Chlorin ug/g	Chlorin/Corg (‰)	THAA (umol/g)	Gly/Ser
<b>Lake Zug North</b>	Plankton	1.57	-	-	-	3191	1.2
	10m	1.10	0.24	166	4.0	1767	1.3
	60m	0.12	0.33	138	0.6	380	1.4
	105m	0.58	0.37	146	0.6	641	1.5
<b>Lake Zug South</b>	Plankton	1.39	-	-	-	3004	1.3
	10m	1.56	0.24	1526	3.9	2620	1.1
	155m	0.89	0.30	79	1.1	405	1.5
	185m	0.96	0.33	66	0.7	519	1.5
<b>Lake Biel</b>	Plankton	1.46	-	-	-	3250	1.4
	10m	0.86	0.32	1197	7.2	693	1.5
	55m	-0.20	0.65	168	2.4	264	1.5
<b>Lake Thun</b>	Plankton	1.29	-	-	-	1220	1.5
	10m	2.04	0.30	790	6.7	435	2.6
	85m	1.80	0.29	85	3.3	110	2.3
	135m	1.48	0.34	47	1.6	88	2.7

Table 1. Plankton and water column parameters for the four lakes

**Table 2:** CW-AA/CP-AA ratio for the four lake basins

	Lake Zug N	Lake Zug S	Lake Biel	Lake Thun
<b>Plankton</b>	1.3	1.3	1.4	1.4
<b>10 m</b>	1.6	1.4	1.8	1.2
<b>middle depth</b>	2.1	1.9	-	1.3
<b>deep depth</b>	2.1	1.9	2.3	1.3
<b>sediment</b>	$2.7 \pm 0.4$	$2.7 \pm 0.2$	$2.7 \pm 0.1$	$2.3 \pm 0.1$

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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