Amino acid and amino sugar compositional changes during in vitro degradation of algal organic matter indicate rapid bacterial re-synthesis

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Abstract

Amino acids (AA) and, more recently, amino sugars (AS) in marine or lacustrine sediments have been increasingly used as paleoproxies. In order to assess AA and AS compositional changes during simulated microbial degradation, as well as to understand the importance of amino-compound re-synthesis by microbes during early diagenesis, decomposition experiments (300 days) were performed with algal (Fragilaria crotonensis) organic matter (OM)/quartz-sand mixtures under controlled redox conditions. Despite expected greater overall degradability under oxic conditions, decomposition kinetics of the bulk algal OM, as well as the total particulate AA and AS were similar under oxic and anoxic conditions, following exponential decay kinetics consistent with the observed mobilization and transfer of large parts of the particulate organic carbon (C) and nitrogen (N) into the dissolved inorganic and organic C and N pools. Carbon-normalized AA and AS yields suggest relative enrichment of amino compounds during partial decomposition, indicating the production and accumulation of microbial biomass during early diagenesis, independent of the redox environment. Moreover, AA and AS compositional changes, such as the relative enrichment of the AA glycine and the AS muramic acid (MurA), and the decrease in the molar ratio of glucosamine and galactosamine (GlcN:GalN) during degradation in both redox systems, were consistent with significant bacterial re-synthesis and the preferential preservation of bacterial biomaterial with increasing diagenesis. Large disparities between different bacterial amino-sugar based estimates of bacterial contribution indicate that bacterial end-member compositions are not currently known well enough to make these bacterial-biomarker constraints quantitative. However, the overall trends are consistent, indicating substantial turnover of eukaryotic into bacterial OM on short time scales of weeks to months. Together these results suggest that the influence of bacterial reworking in conserving sedimentary OM via its transfer into more refractory OM pools may be substantially greater than previously appreciated. We also investigated established amino-compound based indicators of OM degradation, bacterial synthesis, and sediment reactivity. Despite discrepancies, which we attribute to different susceptibilities of the respective indicators towards degradational changes on different time-scales, the tested indices were overall consistent with past data. These results therefore confirm their value as universal indicators of OM diagenesis.

Together, our data highlight the vital role of bacterial reworking on the composition of sedimentary OM, with important

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implications for the alteration of primary geochemical signatures during early sedimentary diagenesis and their use as proxies in paleoenvironmental studies.

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1. INTRODUCTION

Only a small proportion of the organic matter (OM) produced in photic zones of the ocean or freshwater bodies is buried in sediments. Depending on the water depth of an aquatic environment, the major fraction of the OM is degraded through microbial processes in the water column (Ducklow, 2000). In most marine and lacustrine environments, the majority of the OM reaching sediments is remineralized by early diagenetic processes at the sediment-water interface. Sedimentary organic particles are colonized by bacteria that break down OM, releasing dissolved organic compounds and nutrients (remineralization). However, the fraction of OM that escapes complete remineralization to be preserved in marine, estuarine or lacustrine sediments retains substantial information about paleoenvironmental conditions (e.g. Niemann et al., 2012; Wirth et al., 2013). Paleoenvironmental reconstructions are therefore often based on the analysis of the chemical (or isotopic) composition of that fraction of sedimentary OM, which is ultimately preserved, and can constitute a detailed record of variation in past environmental conditions.

However, the preservation of OM is also generally highly selective, because different operational fractions or compound classes have very different susceptibility to microbial degradation (e.g., Harvey et al., 1995; Hedges et al., 1999). Sedimentary diagenesis and bacteria-mediated decomposition can therefore markedly shift the biochemical composition of material ultimately preserved in the sedimentary record (e.g. Cowie and Hedges, 1994; Mollenhauer and Eglington, 2007; Veuger et al., 2012). Understanding the specifics of how OM composition may be altered under different depositional conditions is therefore of critical importance for both paleoceanographic and paleolimnological studies, since the integrity of interpretations derived from both bulk sediment organic composition and specific paleoproxies typically depends on this knowledge. Sedimentary redox conditions are among the most important controls on sedimentary diagenesis, directly affecting bacterial metabolism, and thus both transformation pathways and preservation potential (Sun et al., 2002). In addition, redox-dependent microbial reworking of OM and the biosynthesis of bacterial OM can alter the initial bulk biogeochemical signals (Tremblay and Benner, 2006; McCarthy et al., 2007; Alkhathib et al., 2012), potentially confounding interpretation in terms of surface processes.

Amino acids (AA) and amino sugars (AS) are of particular interest as sedimentary biomarkers, as they are major components of living organisms (e.g. proteins), and represent the large majority of preserved organic nitrogen in recent sediments (e.g., Knicker and Hatcher, 1997), linking together sedimentary carbon and nitrogen cycles. Amino compounds, which compose a large fraction of the OM produced by primary producers, generally decay faster than the bulk OM, making their relative yields in sediment deposits (i.e., %C-AA/%C-AS, %N-AA/ %N-AS) good indicators of the degradation state of the sediments (e.g., Cowie and Hedges, 1994). Moreover, the molecular compositions of these compounds also provide information on OM freshness and diagenetic state (e.g. Cowie and Hedges, 1992, 1994; Benner and Kaiser, 2003; Alkhathib et al., 2012). For instance, the ratio between the AS glucosamine (GlcN) and galactosamine (GalN) decreases with progressing degradation and bacterial reworking of sedimentary OM. Similarly, the ratio of non-protein AA (e.g., β-alanine) and protein AA (e.g., aspartic acid) has been shown to change systematically with the decay and quality of the OM (e.g., Cowie and Hedges, 1994). Based on systematic AA compositional changes during sedimentary diagenesis, Dauwe and co-authors (Dauwe and Middelburg, 1998; Dauwe et al., 1999) proposed an AA-based degradation index (DI) as reliable measure of the degradation history of OM, which has since been widely applied to marine dissolved OM (Davis et al., 2009), marine and estuarine sediments (e.g. Dauwe et al., 1999; Lamstein et al., 2006; Alkhathib et al., 2012), lake sediments (e.g. Meckler et al., 2004), and river systems (e.g. Duan and Bianchi, 2007). Finally, oxic versus anoxic degradation seems to produce significant differences in the molar distribution of AA in degrading OM (Cowie et al., 1995), so that the ratio of specific single AA that become enriched during aerobic decomposition to those that preferentially accumulate under anoxic conditions can be also used to distinguish between aerobic and anaerobic microbial degradation (Menzel et al., 2013).

Microbial communities play a key role as catalytic agents in all of these processes. However, bacteria not only mediate OM decay, but they also add newly produced bacterial biomass to the sedimentary OM pool, thereby changing the bulk biogeochemical composition of the sediments (Lee and Fisher, 1992; Lehmann et al., 2002; Tremblay and Benner, 2006; McCarthy et al., 2007; Alkhathib et al., 2012; Larsen et al., 2015). A number of specific biomarkers have been used to track this process. For example, muramic acid (MurA) forms part of the bacterial cell wall polymer peptidoglycan. It is unique to bacteria (Schleifer and Kandler, 1972) and can therefore be used as a biomarker for living bacteria and recent bacterial necromass (Moriarty, 1975; Benner and Kaiser, 2003; Niggemann and Schubert, 2006; Carstens et al., 2012; Carstens and Schubert, 2012; Niggemann et al., 2018). Similarly for
AS, as noted above, the transformation of planktonic OM into bacterial OM is accompanied by decreasing ratios between GlcN and GalN, which can thus serve as semi-quantitative indicator of sediment bacterial reworking.

While multiple field studies have demonstrated links between organic composition, its degradation state, and the contribution of bacterial remnants to bulk sedimentary OM (e.g. Niggemann and Schubert, 2006; Tremblay and Benner, 2006; Kaiser and Benner, 2008; Carstens et al., 2012; Niggemann et al., 2018), systematic experiments examining molecular-level changes in key biomarkers under oxic vs. anoxic conditions are far more rare. In particular, while initial efforts to establish sedimentary AS as source and degradation indicators have proven promising (e.g. Niggemann and Schubert, 2006; Tremblay and Benner, 2009), there is currently almost no experimental work reporting the systematics of degradation for these compounds. In particular, no studies have examined the importance of oxic vs. anoxic conditions in the change in individual AS during microbial degradation.

Here we performed incubation experiments assessing coupled AA and AS compositional change during simulated early diagenetic degradation of diatom OM (Fragilaria crotonensis) under controlled oxic and anoxic conditions. We assessed the decomposition kinetics of total AA and AS in context of the mobilization and transfer of organic C and N into the dissolved inorganic and the organic C and N pools. Our main objective was to establish robust mechanistic links between the remineralization of OM, its degradation state, and changes in the relative abundance of individual amino compounds during phytoplankton decay. Finally, we also test and evaluate current degradation indices based on AS and AA in our controlled degradations, and explore the use of carbon-normalized yields of AA and AS to characterize the degradation state of the OM. In particular, we examined the contribution of amino compounds from bacterial cell walls to the total AA and AS pool during experimental OM degradation, and assessed their use as indicators of bacterial reworking and for the build-up of bacterial biomass during early diagenesis.

2. MATERIAL AND METHODS

2.1. Incubation experiments

The diatom Fragilaria crotonensis (SAG strain number 28.96; SAG Culture Collection of Algae, Goettingen, Germany) was cultured in a Bacillariophycean-adapted medium (www.epsg.uni-goettingen.de) in 2-L glass culture bottles in 12:12 dark/light cycles at 20 °C. The diatoms were harvested by centrifugation and dried at 20 °C. The dried phytoplankton biomass was mixed with muffled (12 h, 400 °C) quartz-sand (W4, Quarzwerke GmbH) in a ratio of 1:6, with 250 mg of the diatom/sand mixture then weighted into 160 mL serum vials. Water was collected from the meromictic Northern Basin of Lake Lugano (Switzerland). Oxic water was sampled from 10 m depth, and anoxic water from 170 m depth. The water was filtered with glass fiber filters (0.7 μm, Fisherbrand, Germany). The incubation vials were filled with the oxic (140 mL) or anoxic (145 mL) filtered water, and inoculated with 10 mL unfiltered lake water (from 10 m and 170 m water depth, respectively) yielding close to in-situ nutrient concentrations and a natural consortium of bacteria at the beginning of the experiment. No additional nutrients were added.

During the degradations, oxic vials were continuously purged with air, and the anoxic samples were sealed airtight after initial purging with N2. Additionally, oxic and anoxic controls with autoclaved lake water, and controls without particulate matter were set up. All bottles were kept in a shaking incubator (MaxQ 8000, Thermo) at 20 °C and 80 rpm. The duration of the experiment was 300 days. Samples were taken at days 1, 2, 6, 9, 13, 17, 20, 24, 28, 31, 36, 41, 51, 62, 72, 83, 93, 101, 150, 198, 248, and 300. At every sampling point during the degradation experiment, three bottles of each treatment were sacrificed, as well as one corresponding control. For every sample vial the procedure was as follows: First the oxygen concentration was measured directly in the bottle with an oxygen probe (Fibox 3, PreSens), and an aliquot of water (1 mL) was taken and fixed for sulfide analysis with zink acetate solution (1% w/v). Then the complete sample slurry was filtered through a precombusted glass fiber filter with a nominal pore size of 0.7 μm (GF/F, 47 mm diameter, GE Healthcare Whatman). The filters were stored frozen at −20 °C until further analysis of the particulate phase. The filtrate was subsequently passed through a syringe-filter with a pore size of 0.22 μm (PVD0, Roth). Subsamples were taken for dissolved organic carbon and total nitrogen, ammonium (NH4+), and NOx (NO3− and NO2−) measurements. All samples were stored at −20 °C until analysis.

2.2. Chemical analyses

2.2.1. Solute concentrations

Total dissolved organic carbon (TDOC) and total dissolved nitrogen (DN) concentrations were determined on a Shimadzu VCSH analyzer, which was calibrated with potassium hydrogen phthalate (Sigma Aldrich) and potassium nitrate (Merck), respectively. The average analytical error of the method was 9% for TDOC and 1% for DN, respectively. Ammonium concentrations were measured photometrically with the indophenol method. NOx (NO3− + NO2−) concentrations were determined by reduction to NO with V(III), followed by chemoluminescence detection of the NO (Cox, 1980; Garside, 1982; Braman and Hendrix, 1989), with a detection limit of 0.2 μmol/L. In the anoxic samples, we also determined the concentrations of carboxylic acids (e.g. acetate), which are typical intermediates of fermentation processes, using a Dionex HPLC system with an Aminex HPX-87H column (BioRad), 5 mM H2SO4 as eluent, and UV-detection at 210 nm.

2.2.2. Particulate carbon and nitrogen

For the quantification of particulate organic carbon (POC) and nitrogen (PN), sample aliquots were punched out of the filters (i.d. 3 mm), dried at 40 °C, and two of the 3-mm filter discs were placed into tin capsules. The samples were then combusted at 1030 °C in an elemental analyzer (Integra 2, SerCon), and %POC and %PN were
determined by analysis of the purified sample gases CO₂ and N₂. For calibration of the C and N contents, we used an EDTA as standard (41.1% C, 9.6% N). Estimated analytical precision based on replicate sample analyses was ± 0.1% for C and ± 0.4% for N. POC and PN concentrations in the medium were then calculated based on the amount of C and N on the whole filter (extrapolated from the single filter aliquots) divided by the initial volume of water.

2.2.3. Amino acid and amino sugar concentrations

Particulate AA concentrations were determined according to the procedure described in Carstens and Schubert (2012). Briefly, two punched 3-mm sample filter discs were hydrolyzed with 6 M HCl (Merck) for 20 h at 110 °C under N₂ after adding L-norleucine (L-Nle, Sigma) as internal standard. Samples were then dried under vacuum and redissolved in 1 mL 0.01 M HCl. Further processing followed the method of Popp et al. (2007). After purification via cation exchange chromatography (Dowex 50 WX8 resin, 200–400 mesh, BioRad) as described by Metges et al. (1996), and re-acidification of the eluent with 1 mL 0.02 M HCl at 110 °C for 5 min, acidified isopropanol (4:1 isopropanol:acetyl chloride; Sigma Aldrich and Fluka) was added, and samples were heated to 110 °C for 1 h. This esterification step was followed by derivatization with 3:1 CH₂Cl₂:(CF₃CO)₂O (LabScan and Fluka) at 100 °C under N₂ for 15 min to form trifluoroacetic AA esters. For further purification, the samples were dried and re-dissolved in 1:2 chloroform (CHCl₃; Mallinckrodt): phosphate buffer (KH₂PO₄ + Na₂HPO₄; both Fluka), in nanopure water, pH 7). After shaking vigorously, the AA-containing CHCl₃ phase was transferred to a new vial, and the acylation step was repeated to ensure complete derivatization. Finally, the derivatized samples were dried, redissolved in ethyl acetate (EtOAc; Merck) and analyzed on a GC-FID (Shimadzu) equipped with a VF-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness, GL Sciences). The GC settings were as follows: T_injector 180 °C, T_detector 280 °C, GC oven temperature program: 50 °C (held for 2 min), to 110 °C at 8 °C/min, to 125 °C at 2 °C/min, to 200 °C at 4 °C/min, to 210 °C (held 12 min) at 10 °C/min. The flow rate of the carrier gas He was 1 mL/min. For quantification of the derivatized samples, a standard mixture of AA (AA S-18 (Sigma), L-Nle (Sigma), Orn (Fluka), γ-aba (Sigma), β-ala (Aldrich), α-aba (Sigma)) was also derivatized and measured on the GC using the same instrument settings.

Following the same derivatization protocol, D-AA were measured on a Chirasil-L-Val column (25 m × 0.25 i.d., 0.12 µm film thickness; Varian). The GC settings were as follows: T_injector 180 °C, T_detector 280 °C, GC oven temperature program: 80 °C (held for 5 min), to 120 °C (held for 3 min) at 3.5 °C/min, to 152 °C (held for 3 min) at 4 °C/min, to 195 °C (held for 10 min) at 5 °C/min. The flow rate of the carrier gas He was 0.5 mL/min. For quantitative analyses, D-Ala, D-Leu and D-Ser (Sigma) standards were derivatized the same way as the samples, and analyzed on the GC. The concentrations of D-AA were corrected for racemization during hydrolysis following the method of Kaiser and Benner (2005).

For the analysis of particulate AS, filter aliquots (1/4 of the total GF/F filter) were hydrolyzed with 3 M HCl (Merck) for 5 h at 100 °C under N₂. Subsequently, for purification, samples were dried by rotary evaporation and then redissolved in water. After adjusting the pH to 6.6–6.8, the precipitates were removed by centrifugation. The supernatant was freeze-dried, and the sample was further purified by centrifugation after methanol addition (Zhang and Amelung, 1996). After drying the methanol phase under N₂, conversion to aldonitrile derivatives was conducted after Guerrant and Moss (1984). Myo-inositol (Aldrich) was used as internal standard. The derivatized monomers were separated and quantified on a GC-FID system (Shimadzu) equipped with a VF-5 MS column (60 m × 0.25 mm i.d., 0.25 µm film thickness, Varian). The temperatures of the injector and the detector were 250 °C and of 300 °C, respectively. H₂ was used as carrier gas at 2 mL/min, using the following GC oven program: 120–200 °C at 20 °C/min, then to 250 °C at 2 °C/min, then to 270 °C (held 10 min) at 20 °C/min. A mixture of AS standards was also derivatized in parallel with samples, and measured on the GC system for AS quantification. For both AA and AS (as well as for bulk parameters), final concentrations determined for the oxic samples were corrected for evaporative loss during purging (i.e., concentrations were multiplied with the ratio of measured versus initial sample volume).

3. RESULTS

3.1. Particulate organic matter

3.1.1. Decomposition kinetics

Particulate organic matter (POM) concentrations decreased significantly during the course of the incubation experiments, following (at least for POC) expected exponential degradation dynamics (e.g., see Lehmann et al., 2002). Interestingly, both in the oxic and the anoxic incubations PN concentration (but not the POC concentrations) peaked several days after the onset of the incubation experiments. The brief concentration increase above initial values suggests temporary net N transfer from the dissolved into the particulate N pool. With continued degradation, PN concentrations reached relatively stable values after 30 days in the anoxic treatment, and after 50 days in the oxic incubations. The quasi-steady-state concentrations were somewhat lower in the oxic incubations (decrease by 68% with respect to the initial value) compared to the anoxic experiment (decrease by 58%) (Fig. 1). The decrease in POC concentration was even more pronounced in the oxic setting (77% of the initial concentration), whereas in the anoxic setup the concentration decreased only by 52%, suggesting a relatively large POC fraction that is less susceptible to anaerobic versus aerobic degradation. Under anoxic conditions, POC concentrations were more or less invariant already after ~15 days, whereas for the oxic experiment,
apparent steady state was reached only after approximately 50 days, similar to PN.

The degradation kinetics can be described using a double-exponential decay equation, where the POM (POC and PN) can be divided into two fractions that degrade at different rates (Westrich and Berner, 1984; Lehmann et al., 2002):

$$C(t) = a_1 e^{-k_1 t} + a_2 e^{-k_2 t}$$

where $C(t)$ is the concentration of either PN or POC at the time $t$, $a_1$ and $a_2$ represent the PN or POC concentrations of the labile and the more refractory (at the timescale of our incubation experiments) fraction at $t_0$, respectively. $k_1$ and $k_2$ are the decay constants for the more labile and the refractory POM fraction, respectively. The fitted models are plotted in Fig. 1 and the best-fit values for the estimated first-order decay rate coefficients are summarized in Table 1.

Listed are also values for $A_0$, which corresponds to the model-derived fraction of POM that has the lower susceptibility towards microbial degradation (see Lehmann et al., 2002). $A_0$ is a good indicator of the overall degradation potential of organic matter. For both POC and PN, values for $A_0$ were lower under oxic conditions, attesting to the more efficient POM degradation in the oxic setup. With regards to first-order decay constants for the more labile OM fraction, our data indicate that the initial POM breakdown and decomposition occurs more rapidly under oxygen-free conditions.

Inherent to the observed POC and PN trends, C:N ratios of the decaying POM dropped rapidly during the initial degradation phase (first nine days) in both experimental settings from a starting value of 12 to approximately 4 and 5 in the oxic and in the anoxic setup, respectively (Fig. 2). After Day 9, however, the C:N ratio started to increase.

![Fig. 1. Measured POC (closed circles) and PN (open circles) concentrations during the decomposition of *Fragilaria crotonensis* biomass under oxic and anoxic conditions. Error bars represent the standard deviation of triplicate samples. The results of the best fit to the double-exponential decay model are indicated for the total (gray dashed line), the reactive (dotted line), and the slowly-degrading/refractory (black dashed line) POC and PN. The y-axis intercept for the more refractory fractions was used to derive values for $A_0$ (see Table 1).](image)

<table>
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<th>$f$ (%)</th>
<th>$A_0$ (%)</th>
<th>$k_1$ (1/yr)</th>
<th>$k_2$ (1/yr)</th>
<th>$R^2$</th>
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<td>$12.9 \pm 9.0$</td>
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<td>0.61</td>
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<tr>
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<td>51</td>
<td>$71.1 \pm 22.8$</td>
<td>$0.1 \pm 0.1$</td>
<td>0.82</td>
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<tr>
<td>PN</td>
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<td>39</td>
<td>$19.6 \pm 12.5$</td>
<td>0</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Table 1

Decay parameters for degradation experiments: $f$ is the fraction of total POC and PN metabolized after 300 days of incubation, $A_0$ is the double-exponential decay model-derived fraction of less reactive material (see Lehmann et al., 2002), $k_1$ and $k_2$ are first order decay constants for fast and slowly degrading organic matter, respectively.
rather slowly in the oxic incubations and reached values around 9 after 300 days. In contrast, in the anoxic setting after Day 9, the C:N ratio increased rapidly again, after Day 20 reaching C/N values greater than the starting material (around 14–16), and remained with some variability at that high level throughout the remainder of the incubation period.

3.1.2. Amino acid concentration and composition during decay experiments

In the oxic incubations, after the first 20 days, the total particulate AA concentrations decreased rapidly from between ~200 to ~250 μmol/L to concentrations about half of the initial value (Fig. 3). The initial phase of relatively high AA concentrations in the oxic incubation was associated with decreasing C:N ratios (Fig. 2). For the anoxic experiment, the decreasing trend was similar to the one observed in the oxic setup, with an exponential decrease from ~200 to ~100 μmol/L. Overall, in both the oxic and anoxic setups, about half of the total particulate AA had been decomposed by the end of the incubation period (48% and 54%, respectively).

Despite their partial degradation, AA-N and AA-C also became relatively enriched in the OM pool during simulated sedimentary diagenesis. In the starting algal particulate OM, AA accounted for ~16% of the total POC and

Fig. 2. C:N atomic ratio of POM samples under oxic and anoxic degradation. Error bars represent the standard deviation of triplicate samples.

Fig. 3. Particulate amino acid concentrations (single analyses) in the oxic and anoxic incubations.

Fig. 4. Contribution of amino acids to the particulate C (closed symbols) and N (open symbols) pools (%) in the oxic and anoxic incubation.
38% of the total PN (Fig. 4). In both the oxic and anoxic experiments, the relative contribution of AA to the particulate C and N pools, respectively, increased during the first 40 days of the experiment. Thereafter, the percentages were relatively consistent: they decreased again slightly (oxic), and were essentially constant throughout the remainder of the experiment (anoxic). Towards the end of the incubations the average AA contribution to the total POC pool was approximately 30% for the oxic and 20% for the anoxic treatment, respectively. The average AA contribution to the total PN pool was >60% and <50%, respectively, highlighting the differential relative preservation potential of the most refractory pool of AA under oxic versus anoxic conditions.

Changes in molar percentages of individual AA during POM decomposition are shown in the supplementary Fig. S1. In the initial organic algal substrate (*Fragilaria crotonensis*), Glu (glutamic acid), Asp (aspartic acid), Leu (leucine), Lys (lysine) and Phe (phenylalanine) together with Ser (Serine) were the most abundant AA. Glu and Asp remained the dominant AA fractions throughout the incubation period. Given the experimental set up (discrete incubation vials for different time points instead of one single batch incubations) a relatively large variability is not surprising. Yet, over the course of the incubation, some systematic changes of the relative contribution of single amino acids to the total AA pool could be discerned. While the relative fraction of the AA Leu, proline (Pro), Phe, isoleucine (Ile), tyrosine (Tyr) and threonine (Thr) stayed more or less constant, the contribution of Lys and Ser to the total AA pool decreased, and the fractions of Ala and Val increased. Moreover, non-protein AA (i.e., ornithine (Orn), α-aminobutyric acid (α-Aba)) were detected only after Day 17 in both settings (not shown individually). Interestingly, the overall changes in the mol-percentage patterns were quite similar under both redox conditions, except for the relative abundance of Gly, which doubled in the anoxic experiment and increased rapidly to ~17% during the course of both the oxic and the anoxic experiment.

### 3.1.3. Amino sugar concentration and composition during decay experiments

Total AS concentrations decreased in both settings rapidly by 80% within the first day of incubation from ~25 to ~5 μmol/L, but then increased modestly and far more slowly over the next ~20 days, suggesting the synthesis of AS during the production of new microbial biomass (Fig. 5). Initially, algal particulate AS accounted for ~2.3% of the POC and ~4.8% of the PN (Fig. 6). This relative contribution dropped strongly immediately (Day 1), apparently reflecting the strong preferential degradation of particulate AS at the beginning of the incubation. After the initial loss, the AS contribution to the C and N pools increased again (Fig. 6). At the end of the incubation period, the AS contributions to the C and N pools, respectively, were again 2.6% and 4.3% in the oxic, and 2.6% and 3.8% in the anoxic setting.

With >60% of the total AS yield, GlcN was by far the most abundant AS in all samples (Supp. Fig. S2). ManN, which accounted for >30% of the starting algal AS pool decreased dramatically during the first day and contributed always less than 5% thereafter, independent of the redox conditions. The relative abundance of GalN typically showed an increasing trend, most obvious under oxic conditions. The relative abundance of MurA, which was undetectable in the starting material, increased rapidly to ~17% during the course of both the oxic and the anoxic experiment.

### 3.2. Nutrient and dissolved organic matter concentrations

The total DN concentrations were relatively constant during the first 10 days of incubation under oxic conditions, and then increased steadily reaching a 10-fold higher concentration than initially after 150 days (Fig. 7). During the initial phase of the oxic experiment, no NH₄⁺ was detected. Then, transiently, the NH₄⁺ concentrations increased to a maximum concentration of 82 μmol/L on Day 28 before they decreased again below the detection limit after day 31. Parallel with the drop in ammonium, NOₓ concentrations started to rise, reaching >250 μmol/L after 200 days. Thereafter, NOₓ concentrations decreased again.

Under anoxic conditions the DN concentrations increased more rapidly during the first 10 days of incubation, before plateauing (or increasing only slightly) thereafter. Nitrate was not detected throughout the experiment and NH₄⁺ was the main contributor to the dissolved N pool, confirming anoxic conditions throughout the incubation. NH₄⁺ concentrations increased right after the onset of the
experiment, in association with the rapid decay and remineralization of the PN pool, until reaching a plateau at 120 μmol/L after 25 days. The difference between the DN concentration and the inorganic nitrogen fraction (NOx plus NH₄⁺) represents the concentration of dissolved organic nitrogen (data not shown). Throughout most of the incubation, the concentrations of dissolved organic nitrogen were much higher in the anoxic experimental setting (on average 62 μmol/L) than under oxic conditions (on average 28 μmol/L).

In both experimental setups, the DOC concentrations increased drastically (from 150 to 1160 μmol/L in the oxic regime, and from 130 to 1150 μmol/L under anoxic conditions) during the first 48 hours of the incubation period (Fig. 7). In the oxic experiment, the DOC concentrations stayed more or less constant over the following 27 days, and then dropped by approximately 60% to remain relatively invariant, at ~400 μmol/L, throughout the remainder of the experiment. In the anoxic setting, DOC concentrations continued to rise after the rapid increase at the beginning of the experiment, reaching values of ~2300 μmol/L after ~30 days. The relatively constant and high DOC level was maintained until the end of the experiment and corresponds to approximately 40% of the total C pool transferred to the dissolved phase. We did not measure dissolved carbon concentrations other than DOC (i.e., DIC/CO₂/CH₄) in the incubations. Assuming a closed carbon balance, we can approximate, however, from the final residual POC and DOC concentrations that during the anoxic degradation experiment, ~1.6 mmol C/L (or ~25% of the initial POC) were remineralized to DIC (and maybe a minor fraction to CH₄). This compares to ~4.5 mmol/L
remineralized C (or ~70% of the initial POC) in the oxic experiment.

In the anoxic experiment a relatively large part of the DOC derived from short-chain fatty acids. After 6 days of incubation, acetate was detectable and continued to accumulate to maximum concentrations of >600 μmol/L around Day 62 (Supp. Fig. S3). An overall decreasing trend could be discerned afterwards. Yet, acetate concentrations were quite variable, with individual incubation bottles exhibiting continued degradation to low acetate concentrations (78 μmol/L), and others where concentrations remained as high as ~450 μmol/L. Besides acetate, also formate, valerate, and caproate were tentatively identified as fermentation products (data not shown). Their concentrations increased markedly also during the initial phase of the anoxic experiment, but maximum concentrations were lower (~80 μmol/L, ~250 μmol/L and ~350 μmol/L, respectively).

4. DISCUSSION

4.1. Dynamics of bulk organic matter decomposition and dissolved organic matter remineralization

Mineralization of POM is evidenced by the exponential loss of POC and PN and the concomitant increase of dissolved forms of C and N. Although our incubation experiments were run for a longer time period, the final POC (77% and 58% in oxic and anoxic experiments, respectively) and PN (68% and 58%, respectively) degradation was less pronounced compared to batch experiments conducted with lacustrine algae by Lehmann et al. (2002). On the other hand, similar degradation experiments with purified chitin under oxic conditions by Davis et al. (2009) revealed losses of only 44% for POC and of 24% for PN after 30 days of incubation. Comparison of these results underscores findings from Kristensen and Holmer (2001) that the degradability of sedimentary bulk OM is strongly dependent on the structural and chemical composition of the organic components.

Redox conditions have a direct impact on the bacterial metabolism and subsequently on the fate of specific organic compounds (Sun et al., 2002). Several studies on OM degradation have shown more extensive decay under oxic than under anoxic conditions (Harvey et al., 1995; Nguyen and Harvey, 1997; Hedges et al., 1999), confirming the importance of electron acceptors used for OM degradation by organotrophic microorganism, and/or the importance of O2 for the initial enzymatic attack of organic compounds. Similarly in our experiments, fractional C loss was greater under oxic conditions. In a static system like the closed incubation we used for simulating OM degradation in an anoxic environment, oxidants can become limiting. Thus the comparatively large fraction of OM that was not degraded during experimental anoxic degradation (corresponding to the high value for A0; Table 1) might be explained by the fact that substrates relevant for the different fermentation steps and/or necessary electron acceptors may have become limiting (Harvey et al., 1995). However, as was also concluded by Lehmann et al. (2002), the degradation in experimental incubation systems such as used in our study probably simulate quite well the situation in stagnant anoxic freshwater bodies and sediments where oxidants (i.e., NO3, SO42+, etc.) become limited by slow diffusive supply.

The onset of POM hydrolysis and degradation is reflected by a rapid increase in dissolved carbon concentrations both in the oxic and anoxic settings (Fig. 7). In the oxic experimental setup, DOC concentrations stayed constant until Day 28, despite the continuous decay of particulate OM. This highlights that DOC is an intermediate during oxic degradation, which is simultaneously produced during the hydrolytic breakdown of POM, and further remineralized to CO2. The drop in DOC concentrations from Day 31 on corresponds with the time point when essentially all labile POM had been degraded, and further hydrolysis of the more inert POC fraction, and thus resupply of DOC, is relatively slow. The labile DOC was rapidly remineralized to the point where concentrations stayed essentially constant at a relatively low level until the end of the experiment. The remaining DOM fraction reflects refractory DOC that is barely mineralized at the time scale of this degradation experiment. This DOC fraction likely contains significant amounts of bacterial peptidoglycan fragments (see below). Kitayama et al. (2007), for instance, found in incubation experiments with bacterial cells that part of the peptidoglycan released from bacteria represents among the most recalcitrant DOM.

In contrast to the oxic experiment, DOC concentrations in the anoxic experiments increased further after the initial pulse of DOC release (Fig. 7). The lasting increase can be attributed to the continuous liberation of carboxylic acids. These are typical products of the fermentative OM degradation and accumulate when electron acceptors are lacking. These carboxylic acids accounted for a major portion of the DOC in the anoxic experiment (e.g., acetate contributes 17% to the total DOC on average; see Supp. Fig. S3).

During the initial phase (first three days) of the oxic experiments, the total dissolved nitrogen (DN) concentration remained approximately constant, while PN concentrations decreased only slightly (Fig. 7). We hypothesize that the amount of PN being remineralized essentially balanced the fraction of the DN pool that was (re-) incorporated into bacterial biomass. Synthesis of new bacterial biomass is clearly indicated by the transient increase in PN after a week of incubation (Fig. 1). From Day 10 to Day 28, NH4+ accumulates, indicating that its liberation during OM remineralization exceeds incorporation during bacterial biosynthesis and oxidation to NO3. The overall amount of organic DN that accumulated by the end of the experiment was small (<20 μmol/L compared to TN concentrations > 250 μmol/L; data not shown), suggesting that most organic N was mineralized rapidly to NH4+, which is then taken up by bacteria and/or nitrified to NO3.

Under anoxic conditions, the DN concentration increased immediately from the start of the incubation, concomitant with the decrease in particulate N. Nevertheless, as in the oxic incubations, the transient PN concentration peaks (Fig. 1) suggest periods of enhanced bacterial re-synthesis, as discussed in more detail below. The pool of
organic matter (Harvey et al., 1995). Long-term degradation studies with marine organic matter have consistently demonstrated the decrease in carbohydrate and amino acid contents during OM degradation, as well as significant changes in the relative abundances of molecular-level components (Cowie and Hedges, 1992; Skoog and Benner, 1997; Wakeham et al., 1997; Dauwe and Middelburg, 1998). Past studies have suggested a) generally enhanced degradability of carbohydrates and amino compounds relative to the bulk OM, and b) differential remineralization for single compounds within these compound classes. The mol% of AA and AS contents in particular seem to be sensitive to compositional diagenesis (Tremblay and Benner, 2009) (see below).

The diatomaceous substrate used as starting material in the experiments exhibited AA-N% and AA-C% values that fall somewhat into the lower end of the range reported for marine or lacustrine plankton (e.g., Cowie and Hedges, 1992; Carstens and Schubert, 2012), but can still be considered typical for aquatic algae. Similarly, the high contents in Glu and Asp are representative for planktonic OM. In spite of the generally high degradability of AA (e.g., Cowie and Hedges, 1994; Lee et al., 2000), AA concentrations increased initially under both oxygen conditions. Such an increase in particulate AA concentrations was also observed by Harvey et al. (1995) and Nguyen and Harvey (1997) during the experimental degradation of diatoms. The rapid increase in contribution of AA to the bulk N and C pools can likely be attributed to the growth on, and colonization of, the particulate organic diatom debris by bacteria, with rapid bacterial growth producing new amino-compound-rich biomass.

Overall, the concentration loss patterns for individual AA (not shown) were very similar to that of the total AA pool (Fig. 3), yet subtle differences in the degradation of the individual AA led to composition changes with time (Supp. Fig. S1). Under anoxic conditions the relative abundance of Gly increased during the course of the experiment, which is consistent with other longer-term incubation experiments and studies on AA dynamics during diagenesis, which have demonstrated that Gly is an AA that is degraded relatively slowly compared to other AA (e.g., Dauwe et al., 1999; Keil et al., 2000; Veuger et al., 2012). In diatoms, cell walls are enriched in Gly, Ser and Thr (Hecky et al., 1973), suggesting the accumulation of refractory diatom cell wall material in the anoxic incubations. Other explanations for the relative accumulation of Gly may be its association with the refractory bacterial cell wall component peptidoglycan, which is relatively rich in Gly (Madigan et al., 2000), or simply the preferential retention of Gly-rich material during microbial recycling (Vandewiele et al., 2009). It has also been shown in DOM degradation experiments that relative Gly concentration increase can be directly linked to bacterial production rather than selective preservation (Caldeira et al., 2013).

Curiously, in the oxic experiment a similar increase was not observed. Pro in sediments has been reported to have a refractory character similar to that of Gly (Veuger et al., 2012), attributed to its relatively complex molecular structure. However, we did not observe any clear increase in the relative abundance of Pro under either oxic or anoxic conditions. Specific AA molar ratio changes commonly used as indicators of sedimentary degradation state are discussed below (see Section 4.4; Fig. 8). While these were generally consistent with expectations for progressive degradation and microbial reworking, the changes in the overall AA composition were generally subtle in both experimental conditions. More pronounced changes during diagenesis studies have been observed by others (Veuger et al., 2012, and references therein). Differences in the relative compositional changes may be related to the difference in the quality of the matrix/sediments, with different levels of amino-compound protection and binding capacities.

As noted above, the initial AS-C and AS-N (contents 2.4% and 4.8%, respectively) decreased drastically right after the beginning of incubation (Fig. 6). However, in the oxic experiments, the contribution of AS to the C and N pool increased again after the initial drop during the first 30 days of incubation, indicating enhanced AS synthesis. Under anoxic conditions, this effect was less obvious, yet under both redox conditions bacterial turnover (i.e., the transfer of algal OM into bacterial biomass) was indicated by clear compositional changes of the AS pool. The most prominent shift was the large decrease in the molar ratio of GlcN to GalN (from >20 to <10 in the anoxic incubations and <5 in the oxic incubations; Fig. 8e). Comparatively low GlcN:GalN ratios have been reported as characteristic for altered and reworked OM, consistent with an enhanced diagenetic state. Amino-compound based bacterial biomarkers (e.g. the ratio between GlcN and GalN and the fraction of MurA) will be discussed in more detail below.

4.3. Bacterial contribution to the organic matter pool

In aquatic environments heterotrophic bacteria are the primary agents of decomposition and diagenetic alteration of organic detritus (e.g. Benner et al., 1986). They not only metabolize OM, but can add significantly to the bulk OM pool in sinking organic material and sediments in lakes and oceans (e.g., Kaiser and Benner, 2008; Simon et al., 1990; Carstens et al., 2012; Niggemann et al., 2018). With bacterial degradation of labile OM fractions, more refractory material accumulates coincident with formation of new bacterial biomass (Alkhatib et al., 2012). Changes in the elemental composition of the bulk biomass, for example...
its C:N ratio, can be used to track bacterial turnover of OM. Bacteria at maximal growth can exhibit C:N values < 4:1 (Goldman et al., 1987).

Significant contribution of bacteria to the POM pool in our experiment is strongly suggested simply by the rapid decrease in the C:N ratio down to values < 5 at the beginning of the experiment. The later increase in the C:N ratio in both experimental setups might also in part be explained by changes in the growth conditions of the bacteria, as bacterial C:N ratios increase with inhibited growth by limited N supply (Fagerbakke et al., 1996). Limiting growth conditions eventually leads to a net loss of the bacterial biomass by fractional lysis, and hence to a lower contribution of low C/N biomass to the bulk OM pool.

The quantitative contribution of bacterial biomass or necromass to OM has been estimated using a variety of biomarkers that are unique to bacteria (i.e., MurA, D-glutamic acid (D-Glx), D-Ala, etc.). Based on the measured MurA and D-Glx concentrations we applied the approach by Kaiser and Benner (2008) to generate estimates of the fraction of C and N derived from bacteria. This estimate uses the following equation:

\[ \text{Bacterial C(%) or bacterial N(%) = } \frac{\text{BM sample}}{\text{BM bacteria}} \times 100\% \]

where BM sample refers to the measured particulate carbon-normalized yields (or nitrogen-normalized yields) of MurA or D-Glx, respectively, while BM bacteria reflects the average carbon- (or nitrogen-) normalized yield for the biomarker found as reported for cultured bacterial biomass.

The estimated bacterial C(%) and N(%) from this equation are summarized in Table 2. The data show large disparities between MurA- and D-Glx-based estimates, and most of them give unrealistically high predictions for final bacterial contributions for both proxies (e.g., under oxic degradation MurA indicates a maximum bacterial contribution of ~150% for OC and ~260% for TN, while D-Glx indicates ~570% bacterial OC and ~835% bacterial ON). At the same time, however, the relative trends in increasing bacterial contribution between oxic and anoxic degradations are consistent for both MurA- and D-Glx-derived results, indicating substantially faster bacterial turnover and necromass incorporation during oxic conditions.

The lack of plausible quantitative results are likely based on the end member values available, as well as the assumptions inherent in these specific estimates. Calculations of this kind are fundamentally based on two key assumptions: 1) that the end member bacterial biomarker-to-biomass ratios used match those in actual bacteria in the sample measured, and 2) that there is non-selective preservation of bacterial biomass during degradation of the OM mixture, such that the ratios of biomarker/biomass measured in fresh bacteria can be applied directly in preserved/degraded material. Our estimates are based on average BM bacteria yields for freshwater and soil bacteria (e.g., 42.3 nmol MurA (mgC)^{-1}). The results in Table 2 would suggest that in these experiments the bacterial community had a significantly different AS composition, and it is also possible that the AS composition of microbes in the oxic setup differed from that in the anoxic one. The second assumption (constant biomarker/bacterial biomass ratios with degradation) is difficult to assess directly for continuing degradation, however, it seems highly unlikely. A main observation of our overall data is the confirmation of highly
Larsen et al. (2013) show little evidence for bacterial re-frames most analogous to our experiments. Similar amino surface sediments, which would have degradation time- synthesis at least of hydrolysable amino acids in recent the different redox conditions.

Reduced bacterial biomass, and could therefore differ between selectivity during the continuing recycling of newly pro- contribution. However, the degree of overestimation would tion could lead to substantial overestimates of bacterial components of peptidoglycan, which we expect to be selec- with degradation. In particular, D-Glx and MurA are both variable change in specific amino compounds vs. total OM under oxic and anoxic conditions over the course of the degradation experiment, estimated with Eq. (2). Average carbon-normalized yields (42.3 nmol for MurA (mg C)\(^{-1}\) and 61.4 nmol D-Glx (mg C)\(^{-1}\)) and nitrogen-normalized yields (163 nmol for MurA (mg N)\(^{-1}\) and 254 nmol (mg N)\(^{-1}\)) of soil and freshwater bacteria were used, based on data of Kaiser and Benner (2008), Tremblay and Benner (2009), and Bourgoin and Tremblay (2010).

Table 2 Percentages of organic carbon and nitrogen derived from bacteria, calculated based on muramic acid (MurA) and D-glutamic acid (D-Glx) under oxic and anoxic conditions over the course of the degradation experiment, estimated with Eq. (2). Average carbon-normalized yields (42.3 nmol for MurA (mg C)\(^{-1}\) and 61.4 nmol D-Glx (mg C)\(^{-1}\)) and nitrogen-normalized yields (163 nmol for MurA (mg N)\(^{-1}\) and 254 nmol (mg N)\(^{-1}\)) of soil and freshwater bacteria were used, based on data of Kaiser and Benner (2008), Tremblay and Benner (2009), and Bourgoin and Tremblay (2010).

<table>
<thead>
<tr>
<th>Day</th>
<th>Oxic</th>
<th>Anoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% TOC</td>
<td>% TN</td>
</tr>
<tr>
<td></td>
<td>from bacterial cells estimated based on</td>
<td>from bacterial cells estimated based on</td>
</tr>
<tr>
<td></td>
<td>MurA</td>
<td>D-Glx</td>
</tr>
<tr>
<td>1</td>
<td>16.4</td>
<td>115.6</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>186.3</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>182.2</td>
</tr>
<tr>
<td>9</td>
<td>65.4</td>
<td>120.7</td>
</tr>
<tr>
<td>13</td>
<td>40.2</td>
<td>144.2</td>
</tr>
<tr>
<td>20</td>
<td>151.6</td>
<td>176.4</td>
</tr>
<tr>
<td>24</td>
<td>–</td>
<td>255.3</td>
</tr>
<tr>
<td>28</td>
<td>109.7</td>
<td>163.6</td>
</tr>
<tr>
<td>70</td>
<td>–</td>
<td>336</td>
</tr>
<tr>
<td>93</td>
<td>155.1</td>
<td>140.3</td>
</tr>
<tr>
<td>150</td>
<td>–</td>
<td>507.1</td>
</tr>
<tr>
<td>250</td>
<td>98.1</td>
<td>234.8</td>
</tr>
<tr>
<td>300</td>
<td>–</td>
<td>103.9</td>
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<tr>
<td></td>
<td>20.4</td>
<td>61.1</td>
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<tr>
<td></td>
<td>57.4</td>
<td>95.4</td>
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<tr>
<td></td>
<td>–</td>
<td>184.9</td>
</tr>
<tr>
<td></td>
<td>22.7</td>
<td>128.5</td>
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<tr>
<td></td>
<td>36.7</td>
<td>50.9</td>
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<tr>
<td></td>
<td>51.7</td>
<td>155.6</td>
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<td>55.3</td>
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<tr>
<td></td>
<td>60.3</td>
<td>150.1</td>
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<tr>
<td></td>
<td>–</td>
<td>260.4</td>
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<tr>
<td></td>
<td>101.1</td>
<td>–</td>
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<td></td>
<td>–</td>
<td>254.3</td>
</tr>
<tr>
<td></td>
<td>79.2</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>39.2</td>
</tr>
</tbody>
</table>

Clearly, adopting assumed universal values for the biomarker-to-biomass end member ratios and applying the published equations above can, as shown here, generate large overestimates in % bacterial contribution. In particular the disparity between MurA- and D-Glx-based estimates indicate that these estimates must not be consid- ered quantitative. It might be tempting to assume that MurA-based estimates are more accurate (because upper bounds are closer to what appears plausible). However, since both D-Glx and MurA yield non-realistic upper esti- mates, there seems limited basis for this assumption. Another approach might be to normalize the highest values obtained with each parameter, which would then decrease % bacterial estimates at each time point between 1.5 and 8 fold. However, this approach assumes implicitly that bacterial contribution reaches up to 100% in each experiment. Given multiple studies showing that algal remnants and biomarkers are in fact preserved in sedi- ments, this assumption also seems dubious. For example, amino acid isotope studies by Batista et al. (2014) and Larsen et al. (2013) show little evidence for bacterial re- synthesis at least of hydrolysable amino acids in recent surface sediments, which would have degradation time-frames most analogous to our experiments. Similar amino acid isotope preservation patterns have also been observed in lakes (Carstens et al., 2013).

While we conclude that specific bacterial-contribution estimates based on prior equations are clearly not quantita- tive for our system, the data nevertheless consistently indicate substantial and rapid ingrowth of bacterial material under both redox conditions. The increase in the bacterial contribution to the organic C pool with progress of the sim-ulated diagenesis in our experiments is also consistent with observations from Lake Zug. Here, based on MurA anal- yses in sinking OM (from sediment traps) and sediments, Carstens and Schubert (2012) argued that the bacterial con- tribution to the bulk biomass increased with progressing early diagenesis both within the water column and in the sediment, further suggesting that after a short period of time a significant fraction (up to 20%) of the OM had already gone at least once through the microbial loop. Our experimental data confirm, at least qualitatively, the validity of previous assessments of bacterial biomass contribu- tions in natural lake environments, and the use of MurA as reliable tracer of bacterial re-synthesis. Overall, the amino-compound data confirm bacterial turnover was faster under oxic conditions, consistent with the faster micro- biological decomposition of the algal OM.

In agreement with the MurA-based estimates regarding relative bacterial re-synthesis, GlcN:GalN ratios decreased during both incubation experiments towards values of 3 (Fig. 8e), typical for pure heterotrophic bacteria (Benner and Kaiser, 2003; Davis et al., 2009). The decrease in GlcN:GalN from ~20 to 2.6 in the oxic incubation and to 6 in the anoxic incubation thus is consistent with rapid incorporation of algal OM into the bacterial OM pool. With continued diagenesis, C-normalized MurA concentra- tions (as well as GlcN:GalN ratios) plateaued and/or...
decreased only slightly independent of the treatment (Fig. 8e,f). This confirms that the bacterial OM itself can be quite resistant towards further degradation (Ogawa et al., 2001). These observations strongly support the broad conclusion that bacterial reworking is a main mechanism for preserving OM in diverse aquatic environments, as bacterial re-synthesis appears to be associated with the transfer of OM from more labile into rather refractory OM pools.

4.4. Degradation state indices

In environmental samples, a variety of amino-compound-based degradation indices have been developed. For example, carbon- and nitrogen-normalized AA concentrations have been used for the estimation of relative OM degradation state (Cowie and Hedges, 1994; Colombo et al., 1998; Lomstein et al., 2006; Carstens and Schubert, 2012; Alkhatib et al., 2012; Niggemann et al., 2018). Using controlled degradations, this study presents a unique opportunity to verify the validity of amino-compound based OM degradation indicators under contrasted oxygen conditions.

With enhanced degradation, in general the AA-C% and AA-N% have been observed to decrease, because AA containing compounds (e.g., proteins) are usually more susceptible to microbial degradation than other compound groups. However, in our experiments we found somewhat contrasting results, with AA-C% and AA-N% increasing over the course of the experiment. We argue that this is mainly due to the incorporation of AA into refractory bacterial biomass and the preferential degradation of other organic compounds, such as carbohydrates. This interpretation is consistent with the amino-compound compositional changes, indicating that the overall degradation state and bacterial reworking increased during both incubations. Below we consider individually the behavior of widely used amino-compound indicators of relative degradation state.

Relative glycine content: As discussed above, bacterial re-synthesis has a direct impact on the composition of the bulk OM, and the relative abundance of individual AA and AS. This, in turn, can be used for AA-based degradation indices that indicate the relative degree of diagenetic reworking. For example, with early diagenesis Glu, Ile, Leu, Tyr and Phe are likely to be preferentially degraded in sediments (Burdige and Martens, 1988; Lee et al., 2000). On the other hand, glycine (Gly) tends to accumulate relative to other AA due to selective diatom or bacterial cell wall preservation (e.g., Alkhatib et al., 2012), and it has therefore been widely used as a measure of relative degradation state.

However, our degradation data do not follow universal trends with regards to Gly. In our anoxic setup, consistent with field observations, we do see evidence for the accumulation/preferred preservation of Gly towards the end of the incubation (Fig. 8c). In contrast to bacterial cell walls, diatom frustules are enriched in the AA Ser and Thr, as well as Gly (Hecky et al., 1973). The fact that the relative abundance of neither of these two AA increased during simulated algal decomposition suggests that it is not the selective preservation of the algal cell wall material, but rather increased fraction of bacterial necromass, or else the selective removal linked to bacterial degradation, shifting the abundance of all three of these AA. In this regard, our AA data are generally consistent with the AS data, where the time trends for the GlcN:GalN ratios provide evidence for a transfer of carbon from algal to bacterial OM. Overall, however, the relatively modest changes in %Gly we observed may be related to the use of only-diatom biomass for degradation, or else suggest that the timescale of our experiments was not long enough to observe a more prominent increase of this marker.

Non-protein AA: Non-protein AA occur only in trace amounts in living organisms (Cowie and Hedges, 1992). They are mostly formed as metabolic degradation products of proteins. The ratio of a non-protein AA and its precursor can be expressed as RI, or reactivity index introduced by Jennerjahn and Ittekkot (1997). RI, like the DI index discussed below, can be used as an indicator of the relative progress of degradation (i.e., progressive diagenesis is indicated by the increased abundance of non-protein AA).

Our data are consistent with these assumptions, as non-protein AA were near the detection limits during the first month of the experimental period, and measurable amounts appeared only in the second half of the experiment (Supp. Fig. S1). However, the concentrations of the non-protein AA were often near or below detection even after 300 days of incubation, and the reactivity index could only be calculated for some samples (results not shown). This implies that non-protein AA accumulation primarily occurs on longer time scales, and that RI may therefore not be suitable to assess OM degradation at the timescale of weeks to months. This conclusion is consistent with several recent studies. Davis et al. (2009) found that the percentage of non-protein AA is not an effective indicator of diagenetic alteration in the early to intermediate DOM degradation states, and Alkhatib et al (2012) argued that the ratio of protein versus non-protein AA in sediments is pertinent mostly to longer-term degradation (years).

Degradation Index DI: The widely used AA-derived degradation index (DI) is based on a principal component analysis of protein AA mol% values (Dauwe and Middelburg, 1998; Dauwe et al., 1999), and DI values decrease with enhanced degradation. The dynamics of the AA composition changes (and hence the values of the DI) during the oxic decomposition experiment were complex, yet overall they were consistent with this expectation. DI showed a clear drop from <0.8 for the relatively fresh algal material to <0 in the first few days of the experiment, and lower values (despite variability) were maintained through later stages when most of the OM was remineralized (Fig. 8a). In contrast, in the anoxic setting, a very systematic decreasing trend towards low DI values (from 0.7 to ~0.4) was observed (Fig. 8a).

The comparison of the anoxic versus the oxic DI progression suggests that under both conditions AA compositional alteration start essentially immediately during diagenesis. However, at least in our experimental setup, under oxic conditions the initial change was far more rapid, while under anoxic conditions the change was more gradual.
Table 3

Correlations between single degradation indices under oxic and anoxic conditions with significance of correlation (*, p < 0.05; **, p < 0.01; ***, p < 0.001).

<table>
<thead>
<tr>
<th></th>
<th>C:N</th>
<th>%Gly (of Gly+Ser+Thr)</th>
<th>AA/AS</th>
<th>%C-AA</th>
<th>%N-AA</th>
<th>Ox/Anox</th>
<th>DI</th>
<th>GlcN/GalN</th>
<th>GlcN/MurA</th>
<th>%C-AS</th>
<th>%N-AS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Gly</td>
<td>0</td>
<td>−0.37</td>
<td>−0.12</td>
<td>0.18</td>
<td>0.57**</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%C-AA</td>
<td>−0.51*</td>
<td>0.04</td>
<td>0.18</td>
<td>0.3</td>
<td>0.57**</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%N-AA</td>
<td>0.24</td>
<td>−0.05</td>
<td>0.18</td>
<td>0.3</td>
<td>0.57**</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ox/Anox</td>
<td>0.12</td>
<td>−0.19</td>
<td>0.18</td>
<td>0.3</td>
<td>0.57**</td>
<td>0.36</td>
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<td>0.36</td>
<td>0.16</td>
<td>−0.31</td>
<td>0.95***</td>
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<td>GlcN/MurA</td>
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<td>−0.63</td>
<td>0.96*</td>
<td>−0.24</td>
<td>−0.03</td>
<td>−0.22</td>
<td>0.90**</td>
<td>0.79*</td>
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<td>%C-AS</td>
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<td>0.29</td>
<td>−0.91*</td>
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<td>%Gly</td>
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<td>Ox/Anox</td>
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<td>0.47</td>
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<td>0.47</td>
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<td>%C-AS</td>
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<td>0.93***</td>
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and consistent. Previous work (e.g., Davis et al., 2009) has suggested that the DI is a robust index of diagenetic state over longer timescales (decades to millennia). It is possible therefore that over much longer timescales, the results fromoxic and anoxic conditions might converge. However, our data indicate that this index may also be used to indicate diagenetic alteration and OM reactivity changes during early and intermediate degradation stages on shorter timescales, at least under anoxic conditions.

**Amino acid – amino sugar ratios**: The decrease in the ratios of AA:AS under both oxic and anoxic conditions indicates that for decaying algal OM the reactivity of AA is higher than that of AS (Fig. 8d). Considering the variability of behavior of several of the other indices under oxic vs. anoxic conditions, the consistent values and timing of the AA:AS ratio decrease under both redox conditions is somewhat remarkable, and suggests extremely consistent relative reactivity of these biochemical classes, at least for diatom biomass. This observation contrasts with recent reports from a sediment trap study in Lake Baikal (showing an increase in the AA:AS ratio between trap material and sediments; Niggemann et al., 2018), however, it is consistent with observations from shallower lakes in Switzerland by Carstens and Schubert (2012). These authors reported highest AA:AS ratios for fresh plankton and lowest values for the deepest studied sediment layers. The relative lability of AA versus AS may depend strongly on the type of biomass.

Taken together, this study and the data reported by Carstens and Schubert (2012) suggest that at least for algae-dominated OM the AA:AS ratio systematically decreases during OM decomposition, irrespective of oxygen regime. They further suggest that at least during early diagenesis AA:AS represents one of the more universal proxies to diagnose OM degradation. However, at the same time, the fact that AA:AS ratios were essentially invariant under both regimes after Day 30 suggests that their potential to assess diagenetic alteration at a more advanced diagenetic stage is questionable. Overall, our observations are consistent with a relative enrichment in AS in refractory sediments, suggesting that AS are generally more resistant to decomposition during early diagenesis, and confirming their elevated preservation potential relative to AA, independent of the redox state.

**Ox/Anox Index**: As noted above, and based on previous work, the AA molecular-level distribution is expected to change differently during oxic versus anoxic degradation (Cowie et al., 1995). In turn, the Ox/Anox index was introduced by Menzel et al. (2013) as a parameter tracking differential diagenetic AA-compositional changes linked to oxic versus anoxic conditions, specifically to determine the relative importance of aerobic versus anaerobic degradation. This index essentially quantifies the relative enrichment of certain AA during anaerobic degradation (see Menzel et al., 2013). The Ox/Anox ratio in our experiments was on average higher during oxic (2.1) compared to anoxic (1.7) degradation, consistent with Menzel et al. (2013). However, our experimental data did not show any consistent trend of Ox/Anox over time, and the variability was high in both the oxic and anoxic settings (Fig. 8b). In particular the large overlap of values for almost all the time points under both redox conditions suggests that this parameter’s ability to indicate aerobic vs. anaerobic degradation during early diagenesis of algal material is limited.

Overall, the degradation indices determined in this experimental study provided a coherent picture of bacterial degradation of algal OM, consistent with expectations from geochemical studies about diagenetic changes in the molecular composition of lacustrine or marine OM in natural environments (e.g., Dauwe and Middelburg, 1998; Davis et al., 2009; Carstens and Schubert, 2012; Alkhaitib et al., 2012; Menzel et al., 2013; Niggemann et al., 2018). Despite the general agreement of the different degradation state indicators in terms of the quality of a given compositional change of the AA and AS pools, the comparison between the indices (Fig. 8 and Table 3) revealed that they in fact do not always correlate with each other. For example, in the oxic experiment there was a strong correlation between the DI and the GlcN:GalN ratio, while under anoxic conditions, none was observed.

We therefore hypothesize that differential preservation of specific amino compounds, coupled with the accumulation and preservation of bacterial cell-wall remnants, as well as reactivity changes of different OM biochemical classes, result in different degradation indices being applicable to specific stages of diagenesis. A similar idea was previously suggested by Davis et al. (2009) for marine OM and Carstens and Schubert (2012) for lacustrine OM. For example, while we observed most rapid changes in the GlcN:GalN and AA:AS ratios at the very beginning of the experiment, reflecting the rapid initial bacterial turnover, the DI response under anoxic conditions was less immediate, and indicative of a far more steady change in the composition of the AA pool and the reactivity of the residual OM. Indicators based on the relative abundance of non-protein AA, on the other hand, are much less sensitive to diagenetic alteration of algal biomass on the timescale of our experiments, but appear to be good indicators of advanced stages of OM degradation. Together, our data show that none of the indices tested in our study can be considered representative of the full continuum of OM diagenesis. These results strongly suggest that understanding the limitations of each index, in terms of both timescale and oxygen condition is far more important than may be currently recognized.

**5. CONCLUSIONS**

Here we explored the compositional changes of diatom OM during simulated early diagenetic microbial degradation under oxic and anoxic conditions. The decomposition kinetics of total AA and AS during in vitro degradation experiments allowed us to trace the partial mobilization and transfer of organic C and N into bacterial biomass. We specifically tested a wide range of main amino-compound based indicators of diagenetic state on a timescale that is relevant for early diagenesis in natural sedimentary environments, and observed temporal patterns consistent with observations from marine and freshwater settings. Despite different susceptibilities towards degrada-
tion at different time-scales, these all confirm the close links between OM degradation, bacterial synthesis, and a decreasing reactivity of the residual OM, both during oxic and anoxic diagenesis.

Our data highlight that specific bacterial contribution estimates based on bacterial amino sugar biomarker-to-biomass ratios cannot be considered quantitative across all systems. We suggest that careful calibration to specific bacterial sources in given environments could improve such estimates, however, even then quantitative estimates (as opposed to trends) should be viewed with caution. Despite the disparity between MurA- and D-Glx-based estimates, our results nevertheless demonstrate that the bacterial reworking occurs relatively fast, with high OM turnover/re-synthesis already after several weeks (oxic) or months (anoxic). There is growing evidence that the rapid transfer of eukaryotic carbon and nitrogen into bacterial biomass is a general feature of progressing OM degradation, which has important implications for organic geochemical studies that make use of proxy-indicator measurements (e.g., stable C and N isotope measurements of buried algal material to reconstruct paleoenvironmental conditions).

Overall, these new data highlight the importance of understanding the details of diagenetic alteration for the interpretation of any amino-compound proxy used in paleo-environmental studies. The data demonstrate that it is impossible to apply indicators of degradation in a “one size fits all” manner, but that specifics of both redox condition and diagenetic time-scale must always be taken into account when attempting to score degradation. In particular when targeting bulk sedimentary OM in paleo-environmental studies, our study underlines that interpretations must consider that the direct link between the original geochemical signatures produced in the upper water column may be largely decoupled from those ultimately recorded in sediments, as primary signals are likely overprinted by secondary bacterial production during early sedimentary diagenesis.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

MFL and CJS designed research. DC and AD conducted experiments and performed most of the analytical analyses with support from MFL, CJS, and JZ. MFL and DC wrote the manuscript with input from all co-authors.

APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gca.2020.05.025.


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