Metabolism of mineral-sorbed organic matter depends upon microbial lifestyle in fluvial ecosystems

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Key Points:
- Mineral sorption restricts the uptake and mineralization of organic matter by aquatic microbes
- Biofilms and suspended microbial aggregates respond differently to mineral-bound organic matter
- Organomineral sorption alters the stoichiometry of microbial carbon and nitrogen cycling

Supporting Information:
- Supporting Information S1

Correspondence to:
W. R. Hunter,
w.hunter@qub.ac.uk

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Metabolism of mineral-sorbed organic matter and microbial lifestyles in fluvial ecosystems

William Ross Hunter[1,2], Robert Niederdorfer[2,3], Anna Gernand[2], Bart Veuger[4], Judith Prommer[5], Maria Mooshammer[5], Wolfgang Wanek[5], and Tom J. Battin[3]

[1]Queen's University Marine Laboratory, School of Biological Sciences, Queen's University of Belfast, Portaferry, UK, [2]Department of Limnology and Bio-Oceanography, University of Vienna, Vienna, Austria, [3]Stream Biofilm and Ecosystem Research Laboratory, School of Architecture, Civil and Environmental Engineering, Ecole Polytechnique Fédérale Lausanne, Lausanne, Switzerland, [4]Royal Netherlands Institute for Sea Research (NIOZ), Yerseke, Netherlands, [5]Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria

Abstract In fluvial ecosystems mineral erosion, carbon (C), and nitrogen (N) fluxes are linked via organomineral complexation, where dissolved organic molecules bind to mineral surfaces. Biofilms and suspended aggregates represent major aquatic microbial lifestyles whose relative importance changes predictably through fluvial networks. We tested how organomineral sorption affects aquatic microbial metabolism, using organomineral particles containing a mix of ^{13}C, ^{15}N-labeled amino acids. We traced ^{13}C and ^{15}N retention within biofilm and suspended aggregate biomass and its mineralization. Organomineral complexation restricted C and N retention within biofilms and aggregates and also their mineralization. This reduced the efficiency with which biofilms mineralize C and N by 30% and 6%. By contrast, organominerals reduced the C and N mineralization efficiency of suspended aggregates by 41% and 93%. Our findings show how organomineral complexation affects microbial C:N stoichiometry, potentially altering the biogeochemical fate of C and N within fluvial ecosystems.

1. Introduction

Inland waters play a hitherto underappreciated role in the global C cycle [*Battin et al.*, 2008; *Raymond et al.*, 2013]. Streams and rivers receive large deliveries of terrestrial organic C, which is partially metabolized, transiently buried in the sediments, or routed to the coastal ocean where it regulates the cycling of C, N, and phosphorous [*Battin et al.*, 2008; *Bauer et al.*, 2013; *Raymond et al.*, 2013]. One of the least understood fluxes is the burial of organomineral complexes in stable continental deposits such as large floodplains or lake sediments [*Tranvik et al.*, 2009; *Aufdenkampe et al.*, 2011]. Organomineral complexes form through sorption of dissolved organic molecules to mineral surfaces [*Aufdenkampe et al.*, 2001; *Vonk et al.*, 2010]. This is assumed to provide physical protection from microbial degradation by restricting the exposure of organic molecules to attack by extracellular enzymes [*Hedges and Keil*, 1995; *Rothman and Forney*, 2007]. It is, thus, a common belief that organomineral complexation is critical for organic C stabilization. This has led oceanographers and soil biogeochemists to postulate a connection between mineral erosion and organic C burial with implications for the global C cycle [*Berner*, 1989; *Galy et al.*, 2007]. However, it is only recently that organomineral complexation has been recognized as potentially important for the carbon balance at the catchment level [*Aufdenkampe et al.*, 2001, 2011; *Vonk et al.*, 2010].

Microbial life in streams and rivers is dominated by biofilms attached to surfaces or by aggregates (e.g., lake and river "snow") suspended in the water column [*Simon et al.*, 2002; *Battin et al.*, 2003]. Attached biofilms and suspended aggregates harbor abundant, highly active microbial cells embedded in an extracellular matrix and organized along chemical and physiological gradients [*Battin et al.*, 2003; *Wotton*, 2007]. Biofilms and aggregates interact in various ways with minerals and other inert particles. For instance, benthic biofilms in streams sequester and accumulate particles from the stream water [*Battin et al.*, 2003], whereas suspended aggregates in larger rivers and floodplains often form from seeds containing a mineral core [*Simon et al.*, 2002; *Wotton*, 2007]. We currently lack mechanistic understanding of the fine-scale processes that mediate the metabolism of organic compounds bound to minerals in biofilms and aggregates. This is important given the potential implications of organomineral complexation for large-scale biogeochemical cycling especially because global change is predicted to increase the erosion of minerals, organic C and N, and their delivery into streams and rivers [*Quinton et al.*, 2010; *Aufdenkampe et al.*, 2011].

To address the relationship between microorganisms and the biogeochemical fate of organomineral complexes, we experimented with attached biofilms and suspended aggregates and quantified key pathways for organomineral degradation in these microbial lifestyles using dual labeled (^{13}C and ^{15}N) amino acids provided in a free form or bound to organic-free kaolinite particles (<10 μm diameter). Amino acids form a critical component of dissolved organic matter in streams and other aquatic ecosystems because of their intrinsically high bioavailability to microbial heterotrophs [*Volk et al*., 1997]. Kaolinite is a common aluminosilicate mineral that binds organic molecules and affects their transport and metabolism in streams and rivers [*Aufdenkampe et al*., 2001, 2011; *Battin et al*., 2008]. We grew biofilms and microbial aggregates using raw stream water from Oberer Seebach (OSB), Lunz am See, Austria. We then exposed these biofilms and aggregates to fixed amounts of either kaolinite-sorbed labeled amino acids (Min-AA) or dissolved amino acids (DAA), over 7 days. We traced the ^{13}C and ^{15}N into biomass-derived hydrolysable amino acids (HAAs), dissolved organic C and N (DOC and DON), dissolved inorganic C (DIC), ammonium (NH_4^+), and nitrate (NO_3^-). C and N mass balances were closed by calculation of the residual ^{13}C and ^{15}N pools, representing other classes of organic molecules (e.g., carbohydrates and lipids) not detected within this experiment.

2. Material and Methods

Organomineral particles were produced by the adsorption of a ^{13}C, ^{15}N-labeled "cell free" mixture of 20 amino acids (Euriso-Top GmbH, Germany) to kaolinite particles at a 1:5 mass ratio, in ultrapure water (Milli-Q) and maintained at room temperature (24 h) under constant shaking (supporting information Figure S1). The amino acid-kaolinite suspension was adjusted to pH 8 (with 0.1 M NaOH) simulating the water chemistry of the OSB. Particles were harvested, rinsed with ultrapure water (Milli-Q), and lyophilized. This produced organomineral particles containing 0.49 ± 0.04% C (10 atom % ^{13}C) and 0.11 ± 0.01 % N (17 atom % ^{15}N); average particle size was 10 ± 4 μm. Pilot experiments revealed that 18 ± 7% of the initially adsorbed C and 21 ± 8% of the adsorbed N leached from the kaolinite particles over the experimental time scale (see supporting information Text S1).

Benthic biofilms were grown in situ on initially sterile glass slides exposed to raw OSB stream water, over 6 weeks (May–June 2013). Suspended aggregates were formed from the same stream water in sterile bottles mounted on a horizontal shaker. In the laboratory, biofilms on slides were exposed to fixed 204 nmol ^{13}C cm^{-3}; 57 nmol ^{15}N cm^{-3} amounts of either dissolved or mineral-sorbed amino acids, reflecting maximum DOC concentrations within the OSB [*Battin et al*., 1999]. After 30 min, biofilms, the supernatant liquid and any unattached organomineral particles, were gently transferred to precombusted Schott© bottles (100 mL) containing sterile-filtered OSB stream water. As with biofilms, suspended aggregates were amended with either dissolved or mineral-sorbed amino acids in precombusted Schott© bottles. Microcosms were incubated for 7 days at 17°C under an 18:6 light:dark regime (light intensity: 383.5 (±119.80) μmol m^{-2} s^{-1}) mimicking summer conditions in OSB [*Ceola et al*., 2013]. Gentle shaking ensured mixing and reduced sedimentation of the suspended aggregates [*Aufdenkampe et al*., 2001]. Each treatment was operated in four replicates using fixed doses of dissolved and mineral-adsorbed labeled amino acids (10 atom % ^{13}C; 17 atom % ^{15}N). Microcosm oxygen concentrations were monitored throughout the experiment using a PreSens Oxy4 optode system (PreSens Precision Sensing Gmbh, Germany) (supporting information Figure S2).

Microcosms were destructively sampled, with liquid samples taken for determination of DOC, DIC, ammonia, nitrate, and DON concentrations and their respective isotopic ratios. Biofilms were rinsed 3 times with ultrapure water (Milli-Q) to remove excess particulate material, lyophilized, and homogenized. Suspended microbial biomass was harvested by sterile filtration (0.7 μm Whatman GF/F filters); the residue was rinsed 3 times with ultrapure water, lyophilized, and homogenized. Water samples were analyzed to quantify the isotopic ratios ($^{12}C/^{13}C$; $^{14}N/^{15}N$) and concentrations of the DOC (following *Wild et al*. [2010]), DIC (following *Bengtsson et al*. [2014]), and DON NO_3^- and NH_4^+ pools (following *Lachouani et al*. [2010]). Hydrolysable amino acids (HAAs) and the bacteria specific biomarker D-alanine (D-Ala) were extracted from biofilm and suspended aggregate biomass, and their isotopic ratios and concentrations were determined, following *Veuger et al*. [2005, 2007]. From these data we calculated ^{13}C and ^{15}N retention within microbial biomass and the dissolved organic pool, versus mineralization to the DIC, NO_3^-, and NH_4^+ pools (see supporting information Text S1).

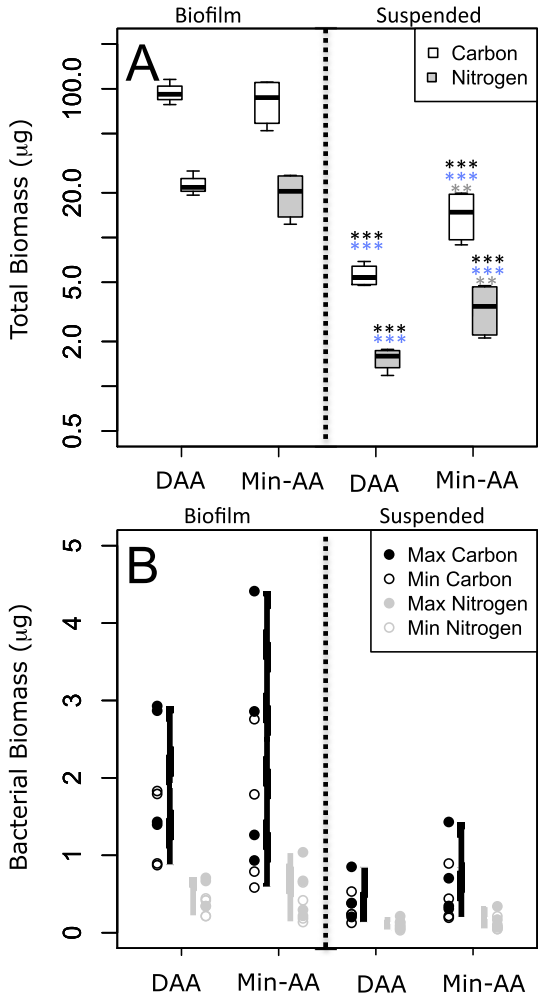

Figure 1. (a) Differences in total biomass C and N in each experimental mesocosm estimated from the total hydrolysable amino acid concentrations. (b) Bacterial biomass estimates for each experimental mesocosm estimated from total hydrolysable amino acid concentrations using the D/L-alanine ratio[17, 18]. Maximum and minimum values for each mesocosm (points) are plotted alongside the treatment-specific ranges (bars). Stars (Figure 1) represent significant pairwise differences identified by two-way ANOVA using the biofilm DAA (black), biofilm Min-AA, (blue), and suspended DAA (grey) treatments as baselines (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Biofilms and suspended aggregates were imaged using epifluorescence microscopy, and vertical oxygen microprofiles were made through biofilms and suspended aggregates at spatial resolutions of 10 to 50 μm [*Berg et al.*, 1998] (see supporting information Text S1).

All data were $\log_{10}$ transformed, and data analysis was conducted using the *base* package in R [*R Development Core Team*, 2009]. We tested for the effects of microbial lifestyle (biofilm versus suspended aggregates) and organic matter source (dissolved versus mineral-bound amino acids) upon total biomass using two-way analysis of variance (ANOVA) and Tukey's honest significant difference. Differences in bacterial biomass were tested using a two-way ANOVA, incorporating maximal and minimal biomass estimates as a random effect. Effects of community organization and organic matter source upon the ^{13}C and ^{15}N budgets and ^{13}C:^{15}N ratios were tested by two-way multivariate analysis of variance (MANOVA). Significant interactions were tested post hoc using pairwise MANOVAs with p values corrected for multiple comparisons [*Benjamini and Yekutieli*, 2001]. Univariate testing (two-way ANOVA) was then performed to identify the individual response variables responsible for differences in each MANOVA model and to test for differences in carbon and nitrogen mineralization efficiency between treatments (summarized in supporting information Table S1).

3. Results and Discussion

Total and bacterial biomass, estimated from hydrolysable amino acid concentrations and D/L-alanine ratios [*Veuger et al.*, 2005, 2007], differed between biofilms and aggregates (Figure 1). Analysis of variance (ANOVA) revealed significant interactions between microbial lifestyle (that is, biofilms versus aggregates) and organic matter source (that is, DAA versus mineral bound) that affected both total C ($p = 0.004$, $F = 12.086$) and N ($p = 0.017$, $F = 8.818$) biomass (Figure 1a). Organic matter source had no effect upon bacterial biomass, while microbial lifestyle represented a significant predictor both in terms of C ($p = 0.004$, $F = 12.461$) and N ($p = 0.004$, $F = 12.091$) (Figure 1b). Biofilms and aggregates had comparable constituents, including diatoms, bacteria, and extracellular substances (Figures 2a and 2c). However, diverging geometries and exposure to the ambient water resulted in differing patterns of oxygen mass transfer in both lifestyles and hence higher respiration rates in the suspended aggregates than in the attached biofilms (Figures 2b and 2d).

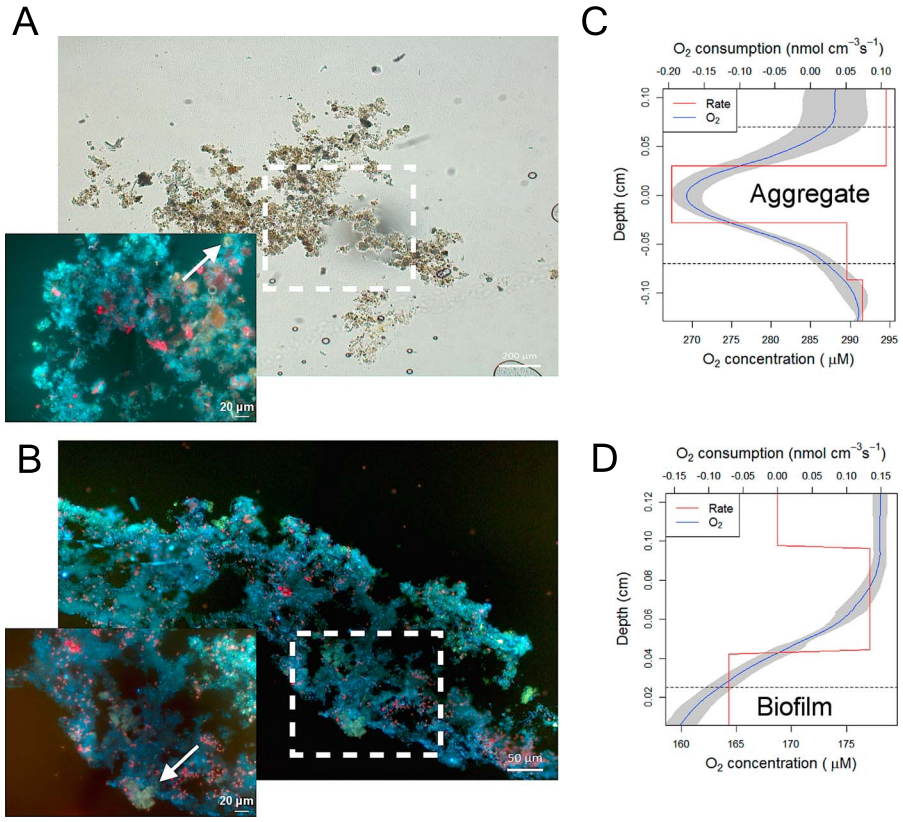

Figure 2. Attached biofilms and suspended aggregates as microbial lifestyles. Cryosections of a (a) suspended aggregate (bright field) and an (b) attached biofilm (epifluorescence microscopy); the arrow denotes incorporated kaolinite in the biofilm; red color refers to chlorophyll *a* autofluorescence. Oxygen profiles and consumption (derived from microelectrodes) differed between (c) suspended aggregates and (d) attached biofilms.

Both microbial lifestyle and organic matter source influenced the fate of C and N within our experiment (C: $p = 0.002$, $_{approx.}F = 11.264$; N: $p < 0.001$, $_{approx.}F = 22.327$) (Figures 3a and 3b). Organomineral sorption significantly restricted C and N mineralization in both microbial lifestyles ($p < 0.01$ in all cases) and their incorporation into the biofilm ($p < 0.01$ in all cases). In biofilms, mineral sorption of amino acids decreased average C and N mineralization by 86 and 96% and incorporation into biomass by 86 and 91%, respectively. In the suspended aggregates, mineral-sorbed amino acids decreased average C and N mineralization by 80% and 99%, while C and N incorporation into aggregate biomass increased by 11 and 167%, respectively. Mineral sorption of the amino acids, therefore, had a significant ($p < 0.001$, $_{approx.}F = 26.438$) effect upon the relationship between C and N use by microorganisms resulting in greater ^{15}N retention in the suspended aggregates than in the attached biofilms (Figure 3c). Consequently, organic matter source represents an important driver of the relative amount of C and N mineralized by each microbial community (mineralization efficiency) (Figure 3d). Specifically, organomineral complexation had differential effects on N mineralization efficiency by the two microbial lifestyles: it reduced biofilm and suspended aggregate N mineralization efficiency by $6 \pm 4\%$ and $93 \pm 2\%$, respectively ($p = 0.001$, $F = 17.246$). There were no effects of microbial lifestyle on carbon mineralization efficiency, which decreased by $36 \pm 15\%$ as a consequence of organomineral complexation ($p = 0.007$, $F = 10.103$).

Supporting our results, both organic matter source and microbial lifestyle affected ^{13}C and ^{15}N retention across the HAA profiles (supporting information Figures S3 and S4). From these compound specific data we infer that unincorporated amino acids represent a minor component of the HAAs within each treatment. By tracing the ^{13}C and ^{15}N labels into microbial HAA pools, we are not measuring microbial incorporation but rather the retention of amino acid-derived C and N within both the cells and extracellular matrices of each community. This is logical, given the potential importance of mineral particles as a nucleation sites for

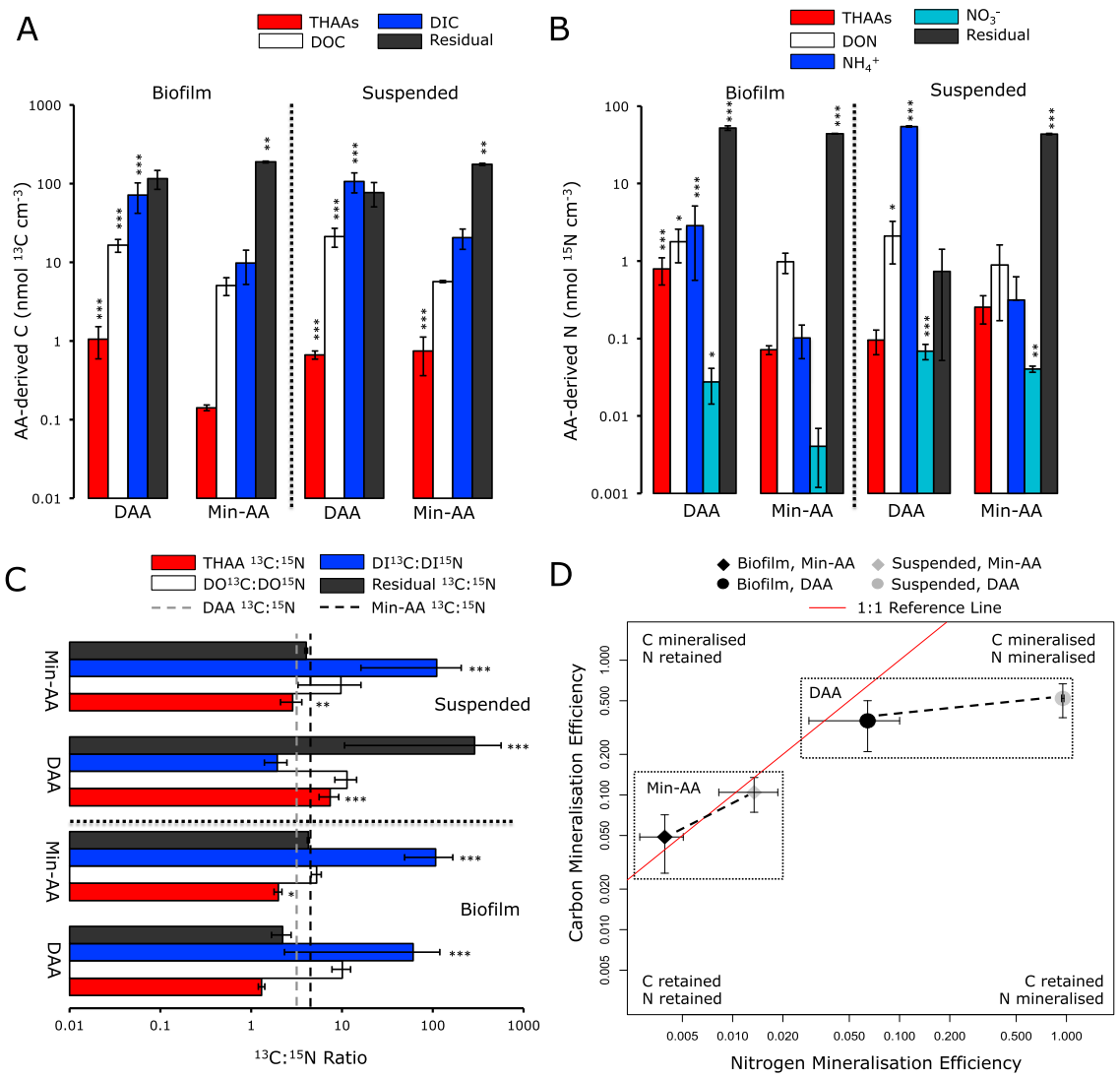

Figure 3. Mean (± standard deviation) dissolved (DAA) and mineral-bound (Min-AA) amino acid-derived (a) ^{13}C and (b) ^{15}N budgets for biofilm and suspended microbial assemblages. (c) Stoichiometric relationships between the ^{13}C and ^{15}N budgets and (d) differences in carbon and nitrogen mineralization efficiency for biofilm and suspended assemblages processing DAA and Min-AA sources. Stars (Figures 3a–3c) represent significant pairwise differences identified by two-way MANOVA using the biofilm DAA (black), biofilm Min-AA (blue), and suspended DAA (grey) treatments as baselines (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

aggregate formation [*Simon et al.*, 2002; *Wotton*, 2007]. Consequently, we discuss our results in terms of amino acid retention within each community, versus mineralization and loss to the residual pool.

These findings provide empirical evidence that organomineral complexes mediate the persistence of organic matter in aquatic ecosystems [*Hedges and Keil*, 1995; *Aufdenkampe et al.*, 2001, 2011; *Vonk et al.*, 2010]. More importantly, we highlight that organomineral complexes differentially affect the mineralization of C and N bound to minerals. This has potential implications for nutrient cycling and ecosystem functioning that requires further investigation at the catchment scale. Amino acids account for up to 10% of organic C and up to 50% of organic N in aquatic systems, where they act as an important microbial dissolved organic matter source [*Hedges et al.*, 1994; *Berggren et al.*, 2009]. Consequently, the stabilization of amino acids through organomineral interaction may have cascading effects in freshwater ecosystems. Polar amino acids such as arginine, histidine, and lysine have multiple amine ($-NH_2^+$) groups and so are relatively N rich. These N-rich amino acids bind up to 3 times more strongly to mineral surfaces than amino acids with hydrophobic side chains (e.g., alanine, leucine, and isoleucine) [*Hedges et al.*, 1994; *Jones and Hodge*, 1999]. Thus, mineral

sorption will have a greater influence upon the bioavailability of organic N versus organic C and likely alter the free amino acid profiles of aquatic systems.

Our findings show that microbial lifestyle alters the stoichiometry of C and N mineralization of organomineral complexes (Figure 3). This is relevant because the relative contribution of attached biofilms and suspended aggregates changes predictably downstream from small headwaters to larger rivers and their floodplains [*Gomi et al*., 2002; *Simon et al*., 2002; *Wotton*, 2007; *Battin et al*., 2008]. In headwaters, the smallest and most abundant streams in fluvial networks, attached biofilms dominate microbial life, while suspended aggregates become more important in downstream ecosystems with elevated residence time and deeper water column. Our findings on oxygen profiles (Figure 2) suggest that attached biofilms behave more like closed systems compared to the suspended aggregates with obvious implications for C and N cycling. In the attached biofilms, C and N may be recycled between algae and heterotrophs [*Battin et al*., 2003], whereas in suspended aggregates microorganisms utilize and release more dissolved C and N to the ambient water [*Simon et al*., 2002]. Bacterial biomass was ~ 4 times lower in the suspended aggregates treatments, yet C and N mineralization was comparable to that in the attached biofilms. This indicates that metabolic demands were greater within the suspended aggregates, which, alongside clear imbalances in the C:N stoichiometry, suggests that C was the primary limiting factor on microbial production in the aggregates. Organomineral sorption of amino acids changes the relationship between aggregate C and N budgets, decreasing relative N mineralization and potentially providing nucleation sites around which microorganisms may aggregate [*Simon et al*., 2002; *Wotton*, 2007]. Consequently, inputs of organomineral complexes may have a greater impact upon the functioning of suspended aggregates compared with attached biofilms.

Organomineral complexation has differential effects upon C and N mineralization efficiencies in biofilms and aggregates (Figure 3d). In the biofilms, changes in C and N mineralization efficiency of 30 and 6% were observed. By contrast, in the suspended aggregate treatments, C and N mineralization efficiencies decreased by 41 and 93%, respectively. In our study, passive desorption accounted for 20% of the mineral-bound amino acids has been made available for microbial degradation. However, desorption of organic matter from mineral surfaces does not represent the only rate-limiting step affecting C and N cycling: rather, organomineral interactions are complex and dynamic. Binding of microbial cells and extracellular enzymes to mineral surfaces synergistically limits amino acid desorption, incorporation, and mineralization [*Dippold et al*., 2014]. Anabolic cellular and extracellular materials are likely to accumulate around the kaolinite particles in an analogous manner to the way microbial polysaccharides and proteins aggregate around mineral surfaces during soil development [*Duemig et al*., 2012]. These products are likely to include more recalcitrant molecules such as long chain polysaccharides and proteins, tannins, and humic substances that bind strongly to multiple sorption sites, ultimately contributing to C and N stabilization [*Miltner et al*., 2009]. This notion is supported by the accumulation of biomass where suspended aggregates processed mineral-bound amino acids (Figure 2a).

Organomineral particles may increase water turbidity and thereby protect free amino acids and DOC from photochemical oxidation [e.g., *Scully et al*., 2003; *Boreen et al*., 2008]. We did not control for photochemical oxidation of microbial responses to changes in light intensity; however, these effects would be consistent between treatments. Thus, the lower C and N mineralization efficiency (Figure 3d) observed in the Min-AA treatments provides evidence that organomineral sorption stabilized AAs against microbial degradation.

Our results suggest that microbial lifestyle plays an important role in regulating the degradation and recycling of mineral-sorbed organic matter. By reducing biofilm C and N mineralization efficiency, organomineral complexes may facilitate downstream transport of organic matter to the lower reaches in a catchment. Here the effects of organomineral sorption have differential effects upon the fate of C and N. Organomineral sorption limited N mineralization efficiency of the suspended microbial aggregates, thereby altering their C:N stoichiometry. Given that the relative importance of biofilms and microbial aggregates changes across fluvial networks [*Battin et al*., 2008], it is, therefore, reasonable to postulate that organomineral complexation will have consequences for carbon and nitrogen fluxes within these systems. We acknowledge that our experiments do not encompass the environmental complexity inherent to fluvial ecosystems. However, we stress that they provide unique mechanistic insights into the fine-scale biogeochemical processes that are required to understand and predict carbon and nitrogen fluxes at catchment scale.

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