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Bioavailability and diagenetic state of dissolved organic matter in riparian groundwater

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[1] Riparian groundwater can exhibit considerable patchiness in the concentration and reactivity of dissolved organic matter (DOM), which ultimately shapes subsurface biogeochemical transformations. Free and combined amino acids are bioavailable constituents of DOM, and their concentration and composition can provide valuable information about the diagenetic state of DOM. Based on riparian groundwater samples and relevant DOM end-member samples, we adapted the amino-acid-based marine DOM degradation index (DI) to groundwater. The groundwater DI was applied to evaluate the spatial and temporal variability in the bioavailability and diagenetic state of riparian DOM in a restored and a channelized section of the River Thur, Switzerland. Among different indicators for DOM diagenetic state (total hydrolysable amino acid concentrations, C-normalized yields, and the contribution of nonprotein amino acids), the groundwater DI correlated best with the activity of the enzyme leucine-aminopeptidase and bacterial secondary production in riparian groundwater. The "freshest" DOM was consistently found in the channel and during high-flow conditions in the groundwater of the restored riparian section and was spatially constrained to a zone inhabited by a dense willow population. The use of amino acid data and the newly developed DI for DOM in groundwater is a promising approach for characterizing the spatial and temporal dynamics of DOM reactivity and diagenesis within riparian groundwater.

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1. Introduction

[2] Rivers represent an important link between terrestrial and aquatic ecosystems, as they ultimately couple biogeochemical cycles of the continents with the marine realm. Dissolved organic matter (DOM) input and bacterial activity in riparian groundwater are highly variable, and microbial decomposition alters DOM composition resulting in DOM that is resistant to further degradation. Hence, characterization of the bioavailability and diagenetic state of DOM is useful for estimating its potential to fuel microbial activity as well as providing insights about biogeochemical pathways.

[3] The river itself presents an important source of DOM to the riparian zone during periods of infiltration. River water is generally a mix of autochthonous and allochthonous DOM, and is typically dominated by terrestrial (allochthonous) components [*Duan and Bianchi*, 2007; *Hedges et al.*, 1994; *McKnight et al.*, 2001]. Along the subsurface flow, interactions with the soil horizon, vegetation, or buried carbon lenses can significantly add organic substrates to the groundwater potentially resulting in a patchiness of DOM availability [*Blazejewski et al.*, 2009; *Clinton et al.*, 2002; *Peter et al.*, 2012a]. Different DOM sources likely differ in their bioavailability and extent of decomposition, which can influence subsurface transformation processes differently, as the extent of OM diagenetic alteration was observed to be inversely proportional to its bioavailability [*Chapelle et al.*, 2009]. For example "fresh" DOM derived from roots of riparian vegetation can accelerate microbial transformation processes leading to the formation of biogeochemical hot spots [*McClain et al.*, 2003; *Schade et al.*, 2001] and a subsequent relative enrichment of less reactive components in the DOM pool [*Benner*, 2003; *Lee et al.*, 2004].

[4] Many marine studies have shown that amino acids are excellent indicators of the degradation state of particulate organic matter (POM) and DOM [*Amon et al.*, 2001; *Dauwe and Middelburg*, 1998; *Davis et al.*, 2009; *Kaiser and Benner*, 2009]. Several indicators of POM diagenetic state have been established based on the percentage of carbon as total hydrolysable amino acids (THAA), the contributions of the nonprotein amino acids β-alanine (β-Ala) and γ-aminobutyric acid (γ-Aba), and a degradation index (DI) based on the composition of amino acids [*Dauwe and Middelburg*, 1998;

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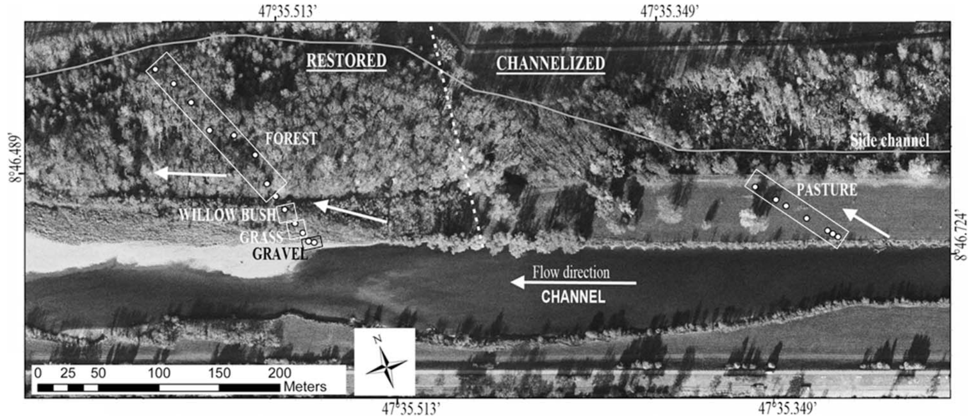

Figure 1. Field site at the River Thur at Niederneunforn, Switzerland, with the nonrestored section represented by the pasture zone and the restored section represented by the gravel, grass, willow bush, and forest zones. River water infiltrates into the riparian aquifer approximately in a northwesterly direction as indicated by the arrows.

Dauwe et al., 1999; *Davis et al.*, 2009; *Yamashita and Tanoue*, 2003]. Hydrolyzable amino acids have also been used to provide insights about POM and DOM reactivity and diagenetic state in rivers and lakes [*Duan and Bianchi*, 2007; *Meckler et al.*, 2004; *Rosenstock and Simon*, 2003]. In groundwater systems, a study by *Chapelle et al.* [2009] showed that THAA concentrations and C-normalized yields provide insights about DOM bioavailability. Nevertheless, a thorough characterization of THAA indicators is still lacking for freshwater systems, and to date no DI has been developed for groundwater DOM. In riparian aquifers, abiotic processes like sorption onto mineral surfaces need to be critically evaluated, because they may play an important role in altering amino acid composition [*Aufdenkampe et al.*, 2001].

[5] In this study, we focused on (1) characterizing the DOM in riparian groundwater of a restored river in terms of its bioavailability and diagenetic state by the use of amino acid concentration and composition and (2) testing the applicability of the DOM DI to groundwater processes. We hypothesized that the application of amino acids in a dynamic riparian aquifer would be useful for identifying hot spots of OM bioavailability and enhancing understanding of OM cycling processes in such systems. The bioavailability and diagenetic state of groundwater DOM was validated with independent data on microbial enzymatic activity and bacterial secondary production measured in the suspended microbial community in the River Thur riparian groundwater [*Peter et al.*, 2012a].

2. Material and Methods

2.1. Study Site

[6] The River Thur originates in the limestone formation of the Mount Säntis region (2500 m above sea level (asl), catchment area: 1700 km^2), crosses the NE Swiss Plateau, and enters the River Rhine at 345 m asl. The River Thur was channelized in the 1890s to protect the river valley against flooding. Since 1993, several 1–3 km long river sections were widened to allow the formation of alternating gravel bars and to increase hydrological connectivity between the main channel and its riparian zone. As a consequence, riparian succession processes were stimulated and habitat diversity increased. The River Thur exhibits a flashy flow regime because reservoirs and natural lakes are absent in the catchment. Maximum, mean, and minimum daily flow rates are 1130, 50, and 2.24 m^3 s^{-1}, respectively, in the recording period 1904–2000 [*Binderheim-Bankay et al.*, 2000].

[7] Instrumented study sites for an interdisciplinary research project on river restoration [*Schneider et al.*, 2011] were located at Niederneunforn (Canton Thurgau, Switzerland) in a 2 km long restored river corridor, and 1 km upstream in a channelized section (Figure 1). In the restored part, we identified four distinct riparian zones across a river-riparian succession gradient that were previously described and monitored for OC dynamics [*Peter et al.*, 2012a]: (1) a bare gravel bank (gravel), (2) exposed gravel colonized by the tall pioneer grass *Phalaris arundinacea* (grass), (3) a second flooding terrace colonized by *Salix viminalis* (willow bush), and (4) the alluvial mixed ash forest (forest) located 50–60 m from the edge of the main channel. At the nonrestored site, no riparian succession is established and pasture makes up one riparian zone. The main channel represents the main source of the infiltrating water [*Vogt et al.*, 2010]. Two piezometer transects were installed in the unconfined aquifer at the restored and the channelized section [*Schneider et al.*, 2011]. The piezometers penetrate the aquifer down to 8 m depth (3 to 7 m screened), allowing water sample collection from the saturated zone, which consists of a deep sandy gravel layer (5–7 m), sealed by an impermeable clay layer at the bottom and covered by a poorly permeable sand and loam layer at the top. The restored study site is characterized by a good hydraulic and hydrological connectivity between river water and riparian groundwater, whereas hydrological connectivity is reduced in the channelized section [*Vogt et al.*, 2010]. The groundwater below the gravel bar and the grass zone is mainly composed of freshly infiltrated river water with ~23 h travel time, while river water reaches the willow bush zone within ~4.5 days, and the forest in the order of weeks.

2.2. Sampling the Riparian Groundwater

[8] Between 2008 and 2009, two sampling campaigns were performed during flood events (April and July 2008) when the river discharge ranged between 240 and 350 m^3 s^{-1}

and four during base to average flow (June and September 2008 and January and April 2009) with discharge of 14–60 m^3 s^{-1}. During these campaigns, the river channel was sampled by directly collecting water with a bottle. Furthermore, the groundwater below the different riparian zones in the restored and the channelized section (Figure 1) were sampled from piezometers using a 12 V purge pump. Prior to collecting groundwater, the electric conductivity and temperature were monitored (WTW, Nova Analytics) to ensure constant values. During the sampling campaign in April 2009, additional soil water samples were taken from the willow bush and grass zones in 0.1, 0.5, and 1 m depth using ceramic suction cups [*Graf Pannatier et al.*, 2011]. Water for DOC analyses was immediately filtered through precombusted (220°C, 4 h) glass fiber filters (GF/F, Whatman). Aliquots of the filtrate were acidified to pH 3 using a 2 mol L^{-1} HCl and stored in muffled glassware at 4°C. The DOC concentration was measured within 1–3 days of sample collection. Aliquots for measuring amino acid composition were stored frozen.

2.3. DOM End-Member Samples

[9] In order to derive the information about the amino acid composition of DOM contrasting in age and diagenetic state, we conducted incubation experiments and sampled an additional aquifer for old groundwater to obtain DOM end-member samples.

[10] Bacterial DOM - Groundwater from the willow bush zone was diluted 1:10 with filtered groundwater (Millipore GSWP, 0.22 μm) and amended with 1 mmol L^{-1} glucose and 10 μmol L^{-1} H_2KO_4P (final concentrations) to ensure high bacterial growth and DOM production. The DOM of the cultures was harvested after 3 days of incubation in the dark by GF/F filtration.

[11] Algal DOM - Periphyton, attached to gravel in shallow areas close to the bank of River Thur, and phytoplankton was collected during an algal bloom in May 2011. They were dominantly composed of C*ladophora sp.*, *Cocconeis placentula*, *Scenedesmus spp.*, and *Monoraphidium sp.* The algae were grown in a beaker with filtered river water (Millipore GSWP, 0.22 μm), and DOM was harvested from the cultures after a growing phase of 3 to 4 days.

[12] Soil and root leachate - Three relatively small plants of *Salix viminalis* were translocated with their original soil from the study site at River Thur to the laboratory. To reestablish stable conditions of the roots and soil sphere, the plants were grown there for two weeks. Subsequently, incubations were set up (1) with willow roots in 1 L high-purity water (Millipore, Milli-Q) for approximately 12 h in the dark, (2) with cut roots in 500 mL high-purity water for 1 h, and (3) with 50 g of soil and associated root material diluted in 200 mL high-purity water for 1 h.

[13] Old and degraded groundwater DOM - A groundwater sample was collected from an artesian well located in the deep Glatt Valley Aquifer (GVA), which was previously described for its old age (>17,000 years) by *Beyerle et al.* [1998]. The glacial aquifer is composed of gravel and sand and confined by poorly permeable Pleistocene sediments.

[14] All DOM end-member samples were GF/F filtered and immediately analyzed for DOC and NO_3^- concentrations. Subsamples for amino acid composition measurements were stored frozen.

2.4. Sorption Experiments

[15] River Thur aquifer material, which was extracted from 2.4 to 3.6 m depth during the well installations on the colonized gravel bar and stored frozen, was used for the sorption experiments. It was composed of coarse gravel to sand/silt material. Prior to incubation it was rinsed three times with high-purity water to limit leaching of DOM from the gravel material, and it was additionally preconditioned for two minutes with the water used for incubation. Triplicate samples of gravel material (~100 mL each) was incubated on a stirrer with 200 mL GF/F filtered water from (1) bacterial cultures, (2) algal cultures, (3) an amino acid standard solution with concentrations corresponding to the average concentration of all river samples, and (4) high-purity water (blank control). The incubation time of 2 h was found to be sufficient for sorption to come to completion [*Ding and Henrichs*, 2002; *Hedges and Hare*, 1987] and short enough to minimize microbial activity. Nonetheless, an additional control experiment on microbial activity was run without gravel material with bacterial- and algal-derived DOM and with the amino acid standard solution. The results from this control were only marginally different from the untreated samples (correlation: $r = 0.976$–0.998) and further used as mol $\%_{\text{initial}}$ in the calculation of the increase and loss of single amino acids (Δmol %) during sorption experiments ($\Delta\text{mol}\ \% = \text{mol}\ \%_{\text{end}} - \text{mol}\ \%_{\text{initial}}$).

2.5. Chemical Analyses

[16] DOC concentrations were measured by using high-temperature catalytic oxidation on a Shimadzu TOC analyzer (TOC-V CPH) [*Benner and Strom*, 1993]. The coefficient of variation among injections of a sample was <1% and blanks were negligible. Dissolved amino acids were analyzed as o-phthaldialdehyde (OPA) derivatives [*Lindroth and Mopper*, 1979] after hydrolysis in 6 M HCl on a HP1100 liquid chromatography system. Samples were hydrolyzed with a microwave-assisted vapor phase technique [*Kaiser and Benner*, 2009]. An Agilent Zorbax SB-C18 (3.5 μm, 4.5 × 150 mm) column with guard at 40°C and a flow rate of 1 mL min^{-1} was used for separation. The linear gradient started from 100% KH_2PO_4 (32 mmol L^{-1}, pH = 7.2) and 0% methanol/acetonitrile (67/33 v/v %) to 48% methanol/acetonitrile after 19 min, and kept at 48% methanol for 2 min. Excitation and emission wavelengths for the detection of fluorescent OPA derivatives were set at 330 and 450 nm, respectively. The limit of quantification ranged from 1 to 4 nmol L^{-1} for individual amino acids. Amino acid analyses were performed in duplicates, and the precision was 5–13%.

2.6. Degradation Index

[17] The original DI was calculated from principal component analysis (PCA) scores described by *Davis et al.* [2009] and *Kaiser and Benner* [2009] for marine DOM using the formula of *Dauwe et al.* [1999] where DI = {[(mol % amino acid − average mol % amino acid) / standard deviation mol % amino acid] × PCA amino acid score}. To develop the DI for river and groundwater, we calculated a new PCA based on the relative abundances of amino acids (mol %) in DOM of the Thur riparian groundwater and the DOM end-member samples contrasting in their age and diagenetic state using the software Simca P+ (12.0.1, Umetrics). We additionally used

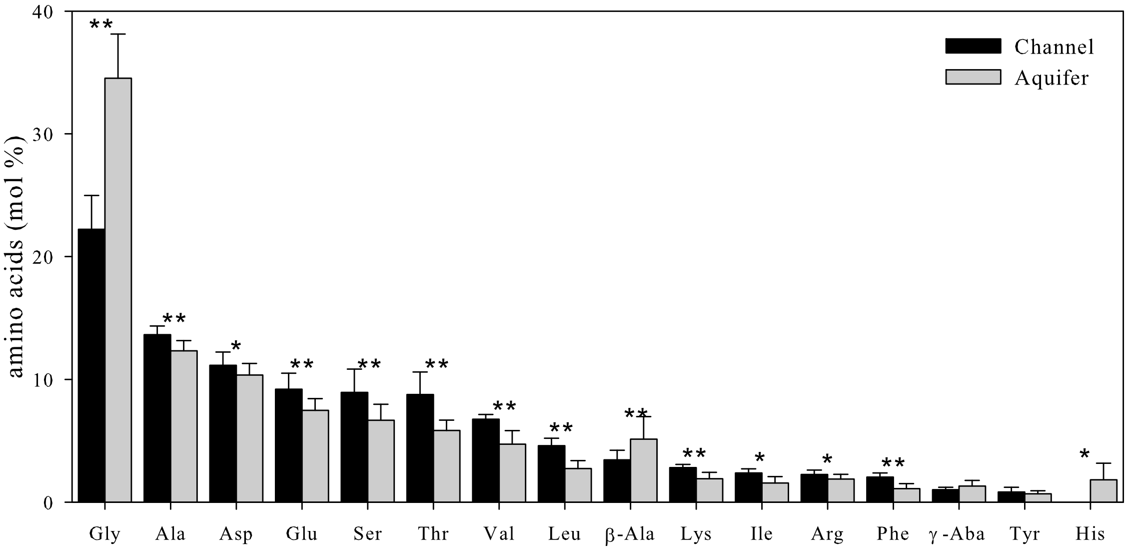

Figure 2. The mean mol % of dissolved protein and nonprotein amino acids for the River Thur channel and riparian groundwater. Stars represent significant (two stars, $p < 0.01$; one star, $p < 0.05$) differences between the mol % amino acids in the channel and riparian groundwater based on nonparametric Mann-Whitney U tests.

the data by *Peter et al.* [2012a] on microbial extracellular enzymatic activity and bacterial secondary production of the suspended microbial groundwater community for correlation analyses with the newly established groundwater DI as well as with the THAA concentrations, the C-normalized yields and the contribution of the nonprotein amino acids.

3. Results

3.1. Amino Acid Composition

[18] In the riparian groundwater of the restored and channelized river sections, glycine (Gly) consistently represented the most abundant amino acid, followed by alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), and serine (Ser) or threonine (Thr) (Figure 2). Compared to concentrations in the channel, Gly exhibited the most striking change (increase) in composition. The other amino acids, except histidine (His) and the nonprotein amino acids β-Ala and γ-Aba, became significantly depleted with respect to the channel.

3.2. Sorption Experiments

[19] We observed minimal THAA removal during the sorption experiments with the bacterial-derived DOM and the amino acid standard solution (Figure 3). For the bacterial DOM, significant loss of Ser and gains in Thr, Arg, γ-Aba, and β-Ala were observed and in the standard solution, losses of Ser and Tyr as well as a gain in Asp were observed. On the other hand, the algal-derived DOM exhibited substantial removal of THAA, but changes in amino acid composition were not consistent compared with the bacterial-derived DOM and the amino acid standard. From experiments with blank water, we observed significant leaching of almost all amino acids from aquifer sediment material, however most pronounced for the amino acids Gly, Ala, Asp, and Glu.

3.3. Groundwater Degradation Index

[20] In order to establish a DI for riparian groundwater, we applied principle component analyses by utilizing groundwater and DOM end-member samples (Figure 4) ranging from very fresh and minimally altered DOM (e.g., algal-derived DOM and root exudates) to very old and reworked DOM (>17,000 year old groundwater). Figure 5 shows the PCA sample scatter and amino acid loadings used to calculate the groundwater DI. The first and the second component extracted explained 31% and 17% of the data variance, respectively. Bacterial-derived DOM scattered in a very distinctive pattern, which can be explained by its high mol % of Val, Ser, and Lys (Figures 4 and 5). Algal-derived DOM exhibited the most positive scores, while the old GVA groundwater showed the most negative scores corresponding to the highest relative contributions of Gly, Phe, β-Ala, and γ-Aba across all end-member samples. The root samples (cut and intact roots were combined as root exudates in Figure 4) scattered very close with the soil leachate samples on the PCA plot with positive scores on the PC1 axis (Figure 5b). The amino acid loadings revealed that Gly had the most pronounced negative effect on the first principle component (PC1), whereas Leu, Ile, Arg, and Lys exhibited the most positive effects (Figure 5 and Table 1). The PC2 was negatively affected by Asp, and positively affected by Val, Lys, and Ser.

[21] A statistical comparison demonstrated that the groundwater DI directly correlates with the marine DOM DI by *Kaiser and Benner* [2009] and *Davis et al.* [2009] when both were applied on the River Thur data set ($r = 0.903$, $p < 0.01$). Both PCA results were similar regards to the loadings of several amino acids (e.g., Gly, Thr, and Arg) on the PC1 axis (Table 1). However, pronounced differences were detected for the PCA loadings of the amino acids Ala, Asp, Ser, and His.

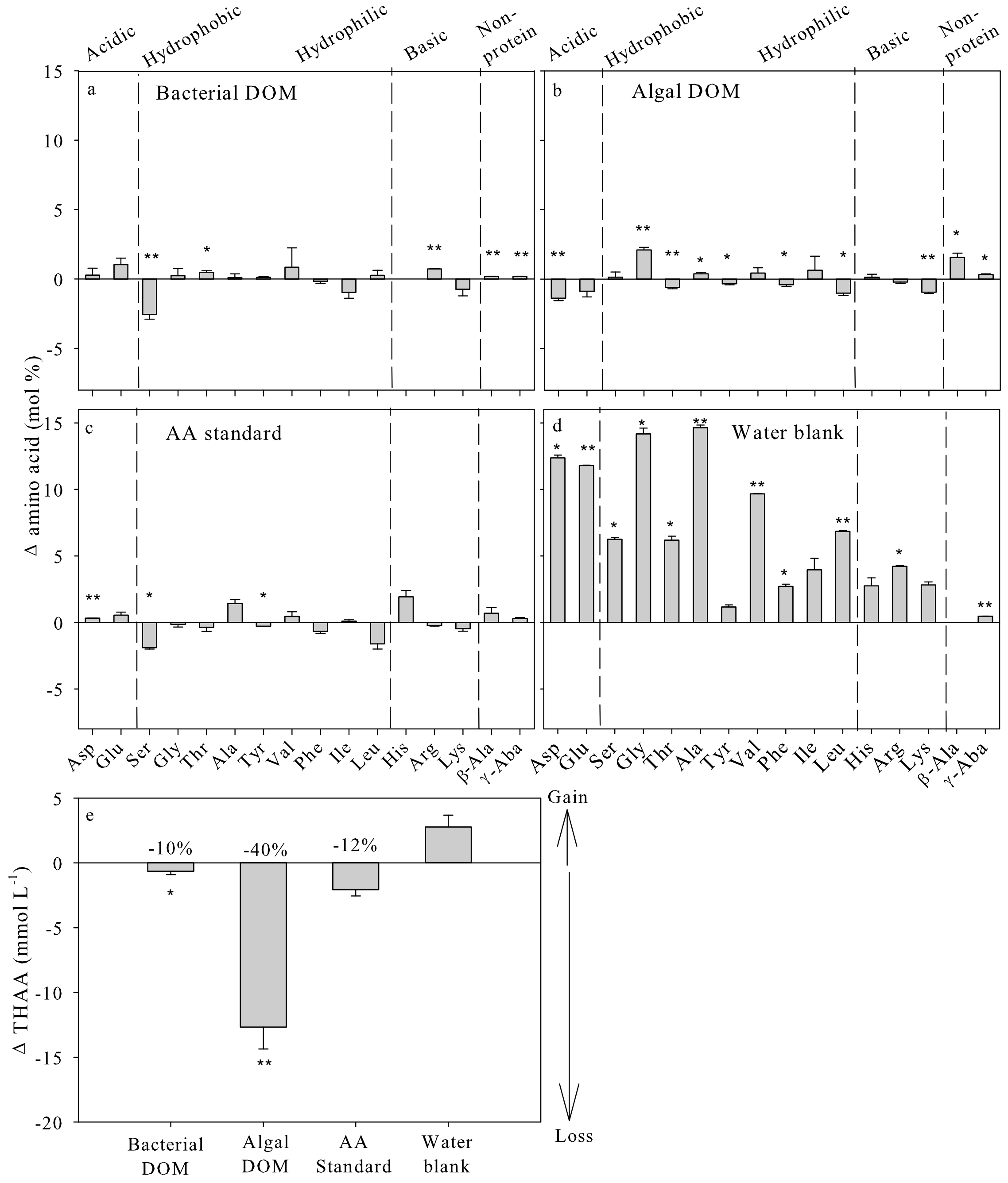

Figure 3. Changes in the mol % of amino acids during sorption experiments with aquifer gravel material (Δmol % = mol $\%_{\text{end}}$ – mol $\%_{\text{initial}}$) for (a) bacterial-derived DOM, (b) algal-derived DOM, (c) the amino acid standard solution, and (d) the water blank. (e) The corresponding concentration changes of THAA during the incubation. Experiments were run in triplicates, and error bars indicate the standard deviation (average error for the water blank). The stars indicate significant differences based on *t* tests (one star, $p < 0.05$; two stars, $p < 0.01$). The *y* axis for Figures 3a–3d represents relative changes (mol %) in contrast to Figure 3e where changes are in concentrations (nmol L^{-1}).

3.4. Different Indicators for DOM Diagenesis and Bioavailability

[22] The DI values calculated for the Thur riparian groundwater samples correlated with the activity of the enzyme leucine-aminopeptidase and with bacterial secondary production (Table 2). Furthermore, the DI values of the different end-member samples were congruent with their assumed respective diagenetic state (Figure 6). Significant correlations were further identified among DOC concentrations, THAA yields and concentrations, and leucine-aminopeptidase activity and bacterial secondary production

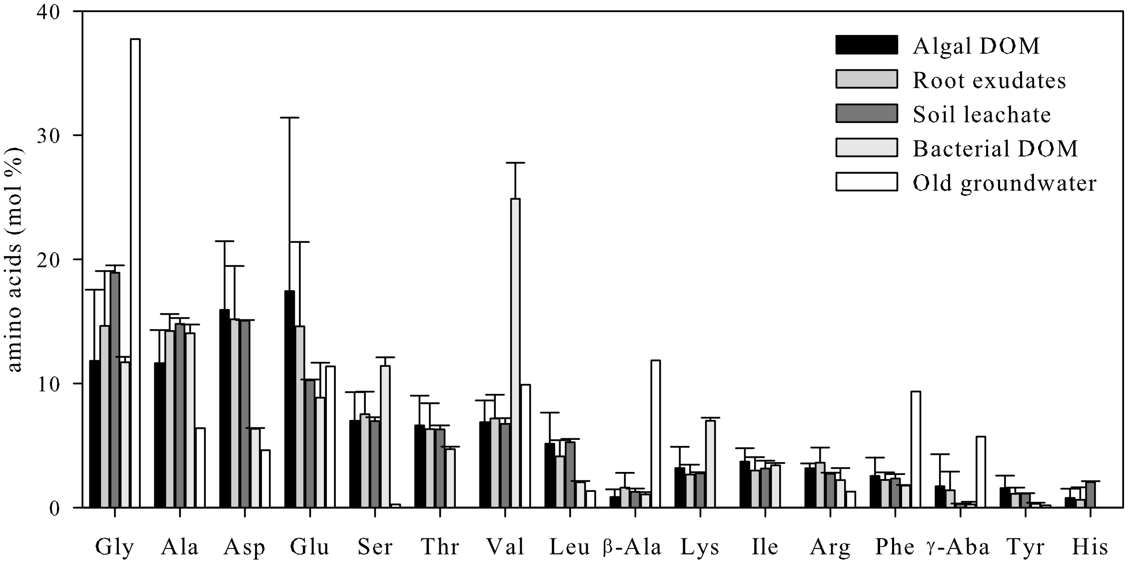

Figure 4. Mol % amino acids for the freshwater DOM end-members. DOM extractions were run in triplicate, and the error bars indicate the standard deviation. The old groundwater was sampled from the Glatt valley aquifer (GVA), and only one sample was available.

(Table 2). We could not match nonprotein amino acids with their respective protein precursor amino acids (Asp:β-Ala and Glu:γ-Aba) by correlation with microbial activity, but there was a weak correlation of β-Ala with the leucine-aminopeptidase activity.

3.5. OM Diagenetic State in the Riparian Groundwater

[23] During base flow conditions, THAA concentrations, C yields, and DI values generally decreased in the riparian groundwater compared to the channel (Figure 7), although they did not exhibit pronounced differences between the individual riparian zones. Flood events completely changed this pattern and caused THAA concentrations and C yields to become elevated in the grass and willow bush zones. In contrast, the forest and pasture zones exhibited low and invariable THAA concentrations and C yields, with no pronounced compositional shifts (DI) during flood events (Figure 7).

4. Discussion

4.1. Amino Acid Composition in the Riparian Groundwater

[24] The hydrolyzable amino acid composition of DOM in the River Thur channel and riparian groundwater was similar

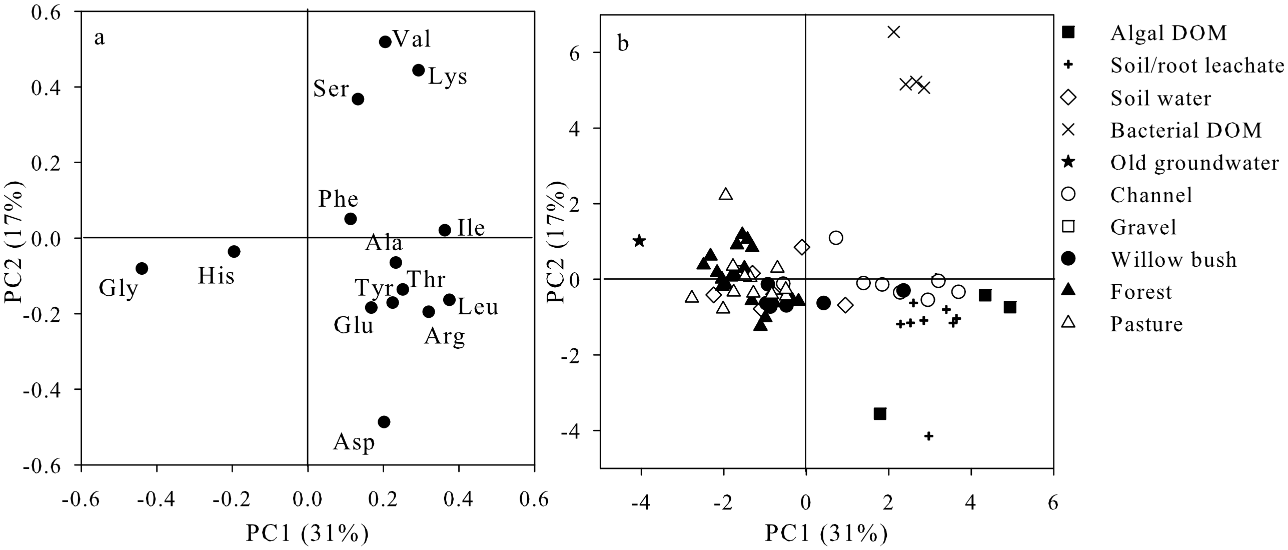

Figure 5. (a) Amino acid loadings plot and (b) sample scatter plot of the PCA derived from the mol % of the individual amino acids of the River Thur groundwater and various freshwater DOM samples. The loadings on the first component (PC1) of the loadings plot (Figure 5a) were used to calculate the new groundwater DI.

Table 1. Parameters Used for Calculation of the New Groundwater DI[a]

	Groundwater DOM			Marine DOM		
	Average mol %	±mol %	PC1	Average mol %	±mol %	PC1
Asp	**10.8**	**1.5**	**0.20**	**11.6**	**2.2**	**–0.04**
Glu	8.2	1.6	0.17	13.2	3.4	0.09
Ser	**7.7**	**2.3**	**0.13**	**6.8**	**2.4**	**–0.01**
His	**1.6**	**1.8**	**–0.20**	**1.2**	**0.8**	**0.06**
Gly	36.5	6.5	−0.44	28.9	8.8	−0.42
Thr	6.2	1.5	0.25	3.7	1.6	0.23
Arg	2.0	0.5	0.32	2.8	2.4	0.31
Ala	**13.1**	**1.2**	**0.23**	**17.0**	**4.2**	**–0.33**
Tyr	0.8	0.3	0.22	1.5	1.1	0.25
Val	5.2	1.3	0.20	1.7	2.2	0.35
Phe	1.2	0.5	0.11	1.5	1.5	0.24
Ile	1.9	0.7	0.36	2.2	1.1	0.22
Leu	2.8	1.1	0.37	1.7	1.7	0.37
Lys	2.0	0.7	0.29	6.1	4.0	0.35

[a]PC1 are the amino acid loadings of the first axes of the respective PCA. The data used for the marine DI are described by *Davis et al.* [2009] and *Kaiser and Benner* [2009]. Bold font indicates the amino acids that deviate strongest between the PCA outputs of the two DOM types.

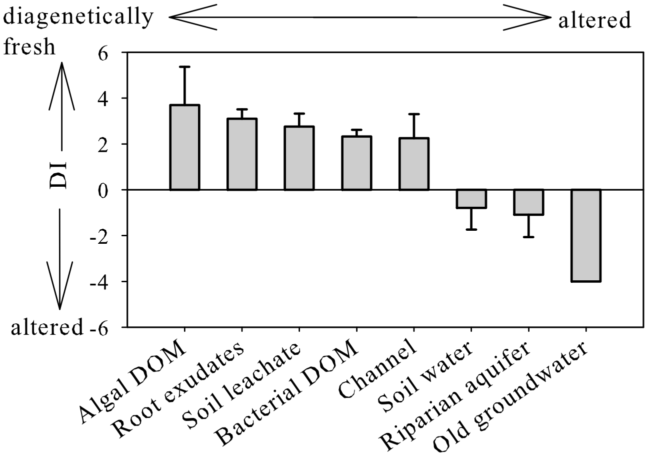

Figure 6. Values derived with the new groundwater DI for the different DOM end-members and the samples from the Thur riparian groundwater.

to compositions reported for other freshwater systems [*Benner and Kaiser*, 2011; *Chapelle et al.*, 2009; *Volk et al.*, 1997]. In the riparian groundwater, most amino acids were significantly depleted in their mol % contribution compared to the channel (Figure 2). Preferential utilization during microbial degradation, resynthesis of proteins by bacteria and sorption and desorption on particulate material can alter the amino acid composition in aquatic systems [*Aufdenkampe et al.*, 2001; *Hedges and Hare*, 1987; *Keil et al.*, 1994]. The infiltration of river water into the subsurface is usually accompanied by intense microbial processing of DOM, which alters its composition and bioavailability [*Sobczak and Findlay*, 2002; *Wong and Williams*, 2010]. Therefore, it is likely that many of the differences in the amino acid composition between the channel and the riparian groundwater were related to microbial processing. Glycine exhibited the most striking increase in composition and can accumulate in groundwater due to its low reactivity, as previously explained by its occurrence in peptidoglycan of bacterial cell walls and intrinsic structural properties of Gly-rich macromolecules [*Kaiser and Benner*, 2009; *Nguyen and Harvey*, 1997, 1998]. The relative abundance of Gly was found to increase during biodegradation experiments [*Benner and Kaiser*, 2011] and to become depleted in the bioavailable components of DOM [*Volk et al.*, 1997]. An increased contribution of the nonprotein amino acids β-Ala and γ-Aba in Thur riparian groundwater compared to the channel further indicates that groundwater DOM becomes more diagenetically altered with infiltration. These amino acids are generally thought to be degradation products and to contribute to the refractory components of organic matter [*Cowie and Hedges*, 1994].

[25] Sorption processes could have produced additional shifts in the amino acid composition in the River Thur aquifer, which is mainly composed of calcareous gravel material [*Gurtz et al.*, 1999]. For example, marine carbonates preferentially adsorb acidic amino acids resulting in high Asp:Gly ratios (up to 2) in sediment OM [*Dauwe and Middelburg*, 1998]. The low average Asp:Gly ratio in all the Thur samples (0.3 ± 0.1) could indicate preferential sorption of acidic amino acids onto the calcareous aquifer material [*Gurtz et al.*, 1999]. However, it might be problematic comparing different systems with respect to sorption processes. Through sorption experiments conducted with the River Thur aquifer material, we could not identify consistent sorption or desorption patterns (Figure 3). Rather they imply differences in sorption processes depending on the source material. Experiments with blank water indicated desorption might also be a considerable process. Overall, sorption processes seem to have inconsistent effects on the groundwater amino acid composition.

4.2. Amino Acids as Indicators for DOM Diagenesis and Bioavailability

[26] This study confirmed that the concentrations of THAA and the amino acid C yields provide useful insights about the bioavailability and diagenetic state of groundwater DOM (Table 2) as previously observed in marine [*Davis and Benner*, 2007] and freshwater DOM [*Chapelle et al.*, 2009]. Furthermore, correlation analyses revealed that DOC concentrations were indicative of bioavailable DOC. However, *Chapelle*

Table 2. Spearman's Rank Order Coefficients (ρ) From Correlation Analyses of the Activity of the Enzyme Leucine-Aminopeptidase (LAP) and Bacterial Secondary Production (BSP), Previously Published by *Peter et al.* [2012a], With Different DOM Parameters Potentially Indicative of DOM Diagenesis and Bioavailability Across All Samples (n > 33) of the Thur Riparian Groundwater

	DOC	THAA	C Yield	Marine DI	Groundwater DI	Asp:β-Ala	Glu:γ-Aba	β-Ala	γ-Aba
LAP	0.45[a]	0.46[a]	0.49[a]	0.57[a]	0.57[a]	−0.24	0.01	−0.35[b]	−0.29
BSP	0.58[a]	0.61[a]	0.39[b]	0.37[b]	0.76[a]	0.14	−0.1	−0.06	−0.02

[a]Here $p < 0.01$.
[b]Here $p < 0.05$.

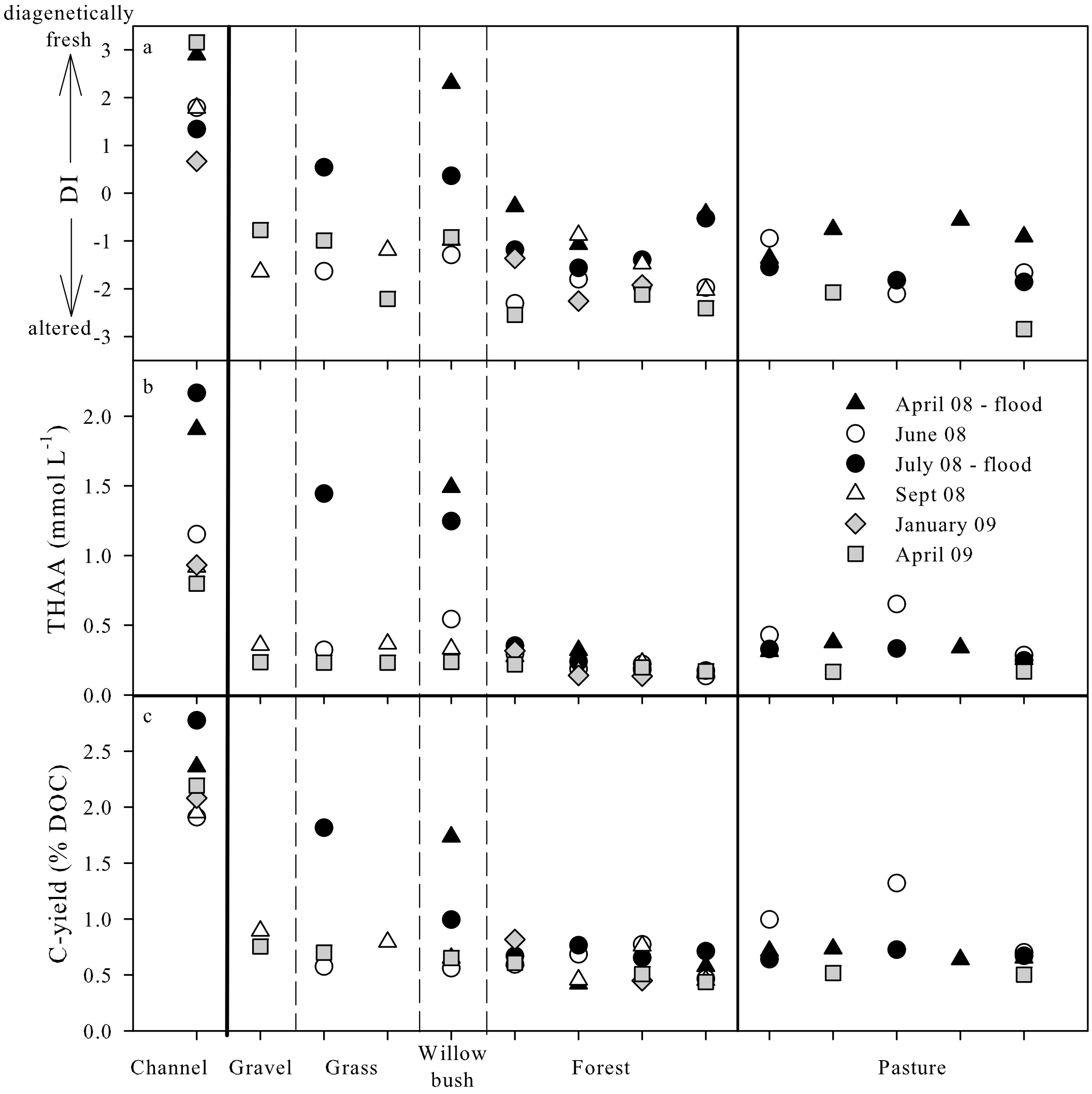

Figure 7. Different indicators for DOM diagenesis and bioavailability: (a) THAA concentrations, (b) C yields, and (c) groundwater DI values for the channel and different zones of the restored and the channelized section (pasture) of the River Thur. Black bold symbols represent sampling campaigns during flood events.

et al. [2009] demonstrated that this assumption is not necessarily valid in other groundwater systems. In contrast to OM in marine sediments [*Cowie and Hedges*, 1994], the ratio of the nonprotein amino acids with their respective protein precursor amino acids (Asp:β-Ala and Glu:γ-Aba) were not useful indicators of diagenesis. However, when investigating the diagenetic behavior of β-Ala and γ-Aba separately, as suggested by *Davis et al.* [2009], there was a weak correlation of β-Ala with the leucine-aminopeptidase activity. Furthermore, *Davis et al.* [2009] demonstrated that nonprotein amino acids are good indicators for the advanced stages of marine DOM degradation. The >17,000 year old Swiss GVA groundwater sample corroborates such a finding, as it exhibits the highest contribution of β-Ala among our end-member samples (Figure 4).

[27] The applicability of the amino acid DI for indicating the DOM diagenetic state in groundwater systems was validated in the present study for the Thur riparian system. The amino acid loadings on the PC1 axis (Figure 5) are usually interpreted to reflect the extent of organic matter alteration [*Dauwe and Middelburg*, 1998]. In accord, the DI values were good indicators for microbial activity and the DI values of the different end-member samples were congruent with their assumed respective diagenetic state (Figure 6). Therefore, differences occurring in amino acid composition in the groundwater samples can be interpreted to represent diagenetic alteration, which directly reflects on the bioavailability of the materials.

[28] Generally, the groundwater DI was similar to the DI for marine DOM [*Davis et al.*, 2009; *Kaiser and Benner*, 2009] with some pronounced differences with regards to the loadings of individual amino acids on the PCA axis (Table 1). For the acidic amino acid Asp, this difference might be explained by enhanced sorption onto the carbonate

aquifer material of the river. Although short-term experiments with fresh OM could not confirm such process (Figure 3), we suspect these experiments might not reflect long-term sorption and desorption processes that could occur during infiltration events lasting several days. The negative scoring of His (Table 1 and Figure 5), indicating a higher contribution in diagenetically altered OM, was rather surprising as this amino acid was reported to be prone to microbial uptake [*Dauwe and Middelburg*, 1998]. However, the study by *Davis et al.* [2009] detected an increase in His during incubation experiments with different sources of marine DOM, indicating that this amino acid can accumulate in degraded DOM. Ala and Ser are both constituents of microbial cell walls, biasing the marine DI to more negative values. From the results of this study it remains unclear why they become selectively depleted in the groundwater amino acid pool. Overall, the marine DOM DI is to some extent applicable to evaluate DOM degradation in freshwater systems. However, infiltration processes can influence diagenetic alteration in a unique way, which makes the use of the new groundwater DI highly advisable for groundwater. The strong correlations between the DI and measurements of microbial activity indicate amino acid compositional shifts can be attributed to microbial rather than abiotic processes. Nevertheless, sorption and desorption processes can affect the contributions of some amino acids, and we should consider that this DI was established for hydrologically well connected glaciofluvial systems and may need some adaptation for systems with a different hydrogeology.

4.3. Spatial and Temporal Variability of OM Diagenetic State in Riparian Groundwater

[29] Amino acid concentrations and compositions were useful for elucidating spatial and temporal patterns in OM bioavailability and diagenetic state of the riparian groundwater (Figure 7). For example, a hot spot for bioavailable OM was observed during flood events in the willow bush zone. The PCA analysis revealed that channel DOM and the soil- and root-derived DOM had similar amino acid compositional patterns. Therefore, DI values for the willow bush flood samples were similar to DI values for the soil and root-derived DOM and the channel DOM (Figure 5). The channel is unlikely to be a source of fresh and bioavailable DOM, because the water residence time during infiltration from the channel to the willow bush zone is rather long (~4.5 days) [*Vogt et al.*, 2010] and bioavailable components can be removed on shorter time scales. Furthermore, the amino acid compositional changes in the willow bush zone were accompanied by increases in DOC concentrations [*Peter et al.*, 2012a]. Hence, the hot spot of microbial activity reported in the willow bush zone during flood events [*Peter et al.*, 2012a] must be supported by bioavailable substrates directly supplied to groundwater below willows. Roots of the riparian vegetation, like those of willow bushes, may constitute a considerable source of fresh and bioavailable DOM [*de Neergaard et al.*, 2002]. In contrast, the forest and pasture zones are less affected by variations of the water table and contain diagenetically altered DOM with low DI values. We conclude that the groundwater DI presented in this study was very useful for elucidating temporal and spatial OM dynamics in riparian systems.

[30] River restoration can affect DOM dynamics in streams by increasing the connectivity between the river, groundwater, soil, and vegetation. The quantity and bioavailability of DOM transported from the root and soil layer to groundwater can increase in a restored section and accelerate microbial transformation of organic carbon [*Peter et al.*, 2012a] and, e.g., nitrogen [*Peter et al.*, 2012b]. This ultimately affects the connectivity between the terrestrial and aquatic realm and how organic carbon and nutrients are transported and cycle through stream systems.

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