

## High DON bioavailability in boreal streams during a spring flood

Ramūnas Stepanauskas<sup>1</sup>

Department of Ecology/Limnology, Lund University, Ecology Building, S-223 62 Lund, Sweden

Hjalmar Laudon

Department of Forest Ecology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden, and  
Department of Environmental Assessment, Swedish University of Agricultural Sciences,  
S-750 07 Uppsala, Sweden

Niels O. G. Jørgensen

Department of Ecology, Royal Veterinary and Agricultural University, Thorvaldsenvej 40,  
DK-1871 Frederiksberg C, Denmark

### Abstract

Riverine inputs of nitrogen is an important factor that controls productivity of coastal marine waters. Dissolved organic nitrogen (DON) comprises most of the N in boreal rivers. During spring floods, DON flux may exceed the baseflow flux by several orders of magnitude. However, little is known about the biological availability of spring flood DON and, thus, its potential effect on coastal productivity.

We have investigated the dynamics of DON bioavailability and chemical composition during a spring flood in two streams in northern Sweden. Potential bioavailability was determined by employing bacterial regrowth bioassays with brackish medium and a coastal bacterial inoculum. In addition, concentrations of urea and amino acids and the proportion of D-isomers in total dissolved amino acids were analyzed because a high proportion of D-isomers is suggested to indicate old and refractory organic material.

During the flood, potential DON bioavailability increased from 19–28% at baseflow to 55–45% during peak flow in the two streams, while DON concentration remained relatively constant. At the end of the flood, bioavailability returned to the baseflow values. Potential DON bioavailability was positively correlated with the concentration of dissolved combined amino acids and the proportion of L-enantiomers of amino acids. However, only 5–18% of DON was identified as urea and free and combined amino acids, suggesting that bacteria also utilized other DON compounds. Our results imply that a major portion of the annual export of labile nitrogen occurred during a few weeks of spring flood.

Because nitrogen usually limits primary production in marine environments (Ryther and Dunstan 1971), riverine input of dissolved organic nitrogen (DON) can be an important factor controlling productivity and eutrophication of coastal waters (Seitzinger and Sanders 1997; Stepanauskas et al. 1999a). Due to a relatively low anthropogenic impact on boreal watersheds, riverine concentration of inorganic nitrogen is usually low, and DON is the dominant nitrogen form (Hedin et al. 1995). In various rivers, 2–70% of DON has been reported to be potentially bioavailable (Seitzinger and Sanders 1997; Stepanauskas et al. 1999a). Spring flood is often the major hydrologic event in boreal rivers, when up to 50% of the annual water discharge may be carried in a

period of a few weeks (Bishop and Pettersson 1996). During snowmelt, water discharge increases by several orders of magnitude, and inorganic solutes are diluted. In contrast to the dilution of inorganic salts, elevated concentrations of dissolved organic matter (DOM) often occur, resulting in an increased transport of DOM (Bishop and Pettersson 1996; Arheimer 1998). This takes place when snowmelt water raises the groundwater level into organic-rich surface soil horizons (Bishop and Pettersson 1996). Furthermore, there are indications that spring flood riverine DOM has elevated nutritional quality (Wikner et al. 1999). Therefore, riverine DOM carried during spring floods may be a major nutrient input to coastal marine waters at high latitudes. However, little is known about the hydrological controls of bulk DOM bioavailability.

In this study, we investigated the dynamics of DON quality during the spring 1998 flood in two streams in northern Sweden. Bacterial regrowth bioassays were employed to track the potential bioavailability of DON throughout the flood event. Brackish water media with a bacterial inoculum from the Baltic Sea was used in the bioassays because (1) this study focused on riverine DON as a potential source of nitrogen to the N-limited southern part of the Baltic Sea; (2) limnic and brackish bacteria may utilize DON to different extents (Stepanauskas et al. 1999a,b); and (3) unified inoc-

<sup>1</sup> Present address: Department of Marine Sciences, University of Georgia, Athens, Georgia 30602-3636 (ramunas@uga.edu).

### Acknowledgments

We are grateful to Benny Brämberg for his excellent assistance running bioassays and Regitze E. Jensen for skillful analysis of amino acids. Roger Wallin, Anneli Sedin, and Pontus Ekman are acknowledged for collecting river water. We thank Lars Tranvik, Lars Leonardson, and Wilhelm Granéli for valuable comments on the paper.

Financial support for the study was provided by MISTRA, the foundation Oscar och Lili Lamms Minne, the Swedish EPA, and the Danish Natural Science Research Council.

Table 1. Catchment characteristics of the investigated streams.

| Characteristic                             | Lillån                                       | Stridbäcken      |
|--|--|------------------|
| Latitude, longitude                        | 63°37'N, 19°42'E                             | 63°30'N, 19°16'E |
| Drainage area (km <sup>2</sup> )           | 11   | 9                |
| Wetland area (%)                           | 25   | 40               |
| Average annual discharge (m <sup>3</sup> ) | ~400   | ~400             |
| Lake area (%)                              | <1   | <1               |
| Forest area (%)                            | 70   | 30               |
| Bare rock/soil (%)                         | 5  | 30               |
| Main vegetation                            | Spruce in lower areas, pine in upper reaches |                  |
| Main geology                               | Migmatite, veined gneiss                     |                  |
| Main soils                                 | Sediment/till                                | Till             |
| Elevation (m above sea level)              | 40–185                                       | 90–220           |
| Stream order                               | 2nd  | 1st              |

ulum, salinity, and pH ensured that observed patterns in potential bioavailability were not caused by differences in these parameters among the water samples.

To determine chemical changes that may cause the fluctuations in potential DON bioavailability, we monitored concentrations of the major identifiable DON compounds during the flood: urea and dissolved free and combined amino acids (DFAA and DCAA, respectively). In addition, the fraction of D-enantiomers in amino acids was analyzed, because a high proportion of D-forms in DCAA is suggested to indicate old and refractory organic material (Pollock et al. 1977; McCarthy et al. 1998; Jørgensen et al. 1999).

## Materials and methods

**Collection of spring flood water**—Two streams in northern Sweden, Lillån and Stridbäcken, were selected for the spring flood study (Table 1). Selection of the streams was based on the following criteria: (1) the streams had low human impact, with no agricultural or liming activity; (2) the catchments had similar size but differed in geology and vegetation; (3) the catchment areas did not include lakes; and (4) previous water chemistry data was available.

Water level was monitored continuously during the spring flood using a pressure transducer connected to a Campbell Scientific data logger. The water level–discharge relationships for the two streams were established previously (Laudon 1999).

Water was collected weekly during baseflow and daily or every other day during high flow, until the discharge returned close to the baseflow level. Immediately after the collection, water was filtered through a 0.2- $\mu$ m pore size filter (SuporCap 100, Gelman Sciences) and stored in acid-washed polyethylene flasks at 4°C until the end of the sampling period. After the storage period (up to 2 months), bacterial abundance in the water was below the detection limit (<10<sup>7</sup> L<sup>-1</sup>). About half of the collected samples were analyzed for inorganic cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup>) and dissolved organic carbon (DOC). Six samples from each stream were selected for DON bioavailability determination and 10 samples per stream were analyzed for urea and amino acids. The

samples were chosen to cover the periods of winter baseflow, initiation of flood, peak flow, and return to baseflow.

**DON bioavailability assays**—The potential bioavailability of DON was determined employing N-limited bacterial re-growth cultures according to Stepanauskas et al. (1999a). Nine hundred milliliters of river water was mixed with 125 ml of artificial sea water (according to Guillard [1975], final salinity 8.3‰), and pH was adjusted to 8.0 by titration with NaOH. To ensure that no phosphorus or carbon limitation occurred, 20  $\mu$ M Na<sub>2</sub>HPO<sub>4</sub> (final concentration [concn.]) and 138  $\mu$ M glucose (final concn.) were added to the cultures. Each medium was filtered through a 0.2- $\mu$ m pore size Supor filter (SuporCap 100, Gelman Sciences) and distributed into eight polystyrene flasks, 125 ml each. Four of the flasks received nitrate spikes (3.82  $\mu$ M N, final concn.). The media were inoculated by adding 6 ml of filtered (Whatman GF/F glass fiber filter, to remove bacterivores) water from the Baltic Sea at Stenshuvud (55°39'N, 14°16'E), with 8 psu salinity and pH 7.8. Incubations were performed at 20°C in the dark for 14 d to ensure that bacteria reached stationary growth phase. Samples for determination of cell abundance were taken at 24-h intervals, fixed with 2% borax-buffered particle-free formaldehyde (final concn.), and stored refrigerated. Cell counts were performed within a month after sampling. After microscopic examination, no bacterivores were found in any of the cultures.

From each replicate culture, only the highest bacterial abundance reached was used for DON bioavailability calculation. First, nitrogen content of an average cell ( $N_{\text{cell}}$ ) was calculated as  $N_{\text{cell}} = s / (D_{\text{spike}} - D_{\text{river}})$ , where  $D_{\text{spike}}$  was the average cell abundance in cultures with riverine nitrogen and nitrate spikes,  $D_{\text{river}}$  was the average cell abundance in cultures with riverine nitrogen only, and  $s$  was the concentration of the nitrate spike. Concentration of potentially bioavailable DON ( $b$ ) was estimated as  $b = N_{\text{cell}} \cdot D_{\text{river}} - i$ , where  $i$  is the concentration of inorganic nitrogen in the medium. The estimated N content per cell was  $2.28 \pm 0.28$  fmol (mean  $\pm$  SE), which was similar to the values ( $1.89 \pm 0.33$ ) from a similar study by Stepanauskas et al. (1999a). Because of insufficient analytical sensitivity of DON measurements, we did not directly monitor DON concentration decline in the cultures.

Estimation of  $N_{\text{cell}}$  and DON bioavailability by using nitrate spikes involves several assumptions, scrutinized by Stepanauskas et al. (1999a). The main assumption is that different bioavailable N compounds yield identical cell yields. In support of this, Stepanauskas et al. (1999a) found no difference in cell yield after additions of nitrate and ammonium. Furthermore, there was no difference in the cell size of bacteria cultivated on DON substrates alone and with nitrate spike added. This is not surprising, since cell size and  $N_{\text{cell}}$  determinations are performed in stationary phase cultures. In all cultures, inorganic nitrogen is depleted during the first few days. After that, media in cultures with and without spikes become identical, probably resulting in similar microbial assemblages. However, bacterial assemblages in bioassays most likely are different from natural assemblages because of the lack of grazing pressure and light, altered temperature and substrate composition, and nutrient addi-

tions. Therefore, the obtained bioavailability values should be viewed as an estimate of the potential bioavailable fraction of DON.

**Measurements of bacterial abundance**—Bacterial abundance was measured using a flow-cytometric method (del Giorgio et al. 1996; Stepanauskas et al. 1999a; Bertilsson et al. 1999). Syto 13 stain (50  $\mu\text{M}$ , Molecular Probes) and Fluoresbrite Carboxy YG microspheres (1.58  $\mu\text{m}$  diameter,  $\sim 3 \times 10^5 \text{ ml}^{-1}$ , Polysciences) were added to 1-ml subsamples and analyzed with a Becton Dickinson FacSort flow-cytometer at a low sample flow rate ( $\sim 12 \mu\text{l min}^{-1}$ ). The cytometer was controlled with CellQuest 1.2 software. Bacterial cells and microspheres were separated in a log-log scattergram of green fluorescence intensity (FL1) and side scattering (SSC). Voltages for these parameters were set to 560 and 400, respectively. Samples were run for 1 min or until 10,000 cells were counted. Bacterial abundance in the samples was calculated using microspheres as an internal standard. The abundance of the microspheres in a stock solution was analyzed by epifluorescence microscopy on a weekly basis.

**Chemical analyses**—Before analysis, samples for DOC were stored frozen in precombusted glass vials with Teflon lids. For other analyses, samples were stored frozen in acid-washed plastic vials. DOC was analyzed after acidification and purging of inorganic carbon by the Pt-catalyzed high-temperature combustion method using a Shimadzu TOC-5000 total carbon analyzer. Standard methods were applied to measure nitrate plus nitrite (Wood et al. 1967) and ammonium (Chaney and Marbach 1962). Total nitrogen was measured with an ANTEK 9000 high-temperature combustion total nitrogen analyzer. Organic nitrogen concentrations were calculated by subtracting ammonium, nitrate, and nitrite from the total N. Urea was measured according to Price and Harrison (1987). Base cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$ ) were analyzed by an absorption spectrophotometer.

DFAA and DCAA were quantified as fluorescent *o*-phthalaldehyde (OPA) derivatives by high-performance liquid chromatography (HPLC) according to Lindroth and Mopper (1979) and Jørgensen et al. (1993). The following individual amino acids were detected: aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala),  $\gamma$ -aminobutyric acid (GABA), tyrosine (Tyr), methionine (Met), valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), ornithine (Orn), and lysine (Lys). DCAA were hydrolyzed to individual DFAA by a microwave technique (Jørgensen and Jensen 1997). To convert molar concentrations of amino acids to moles of carbon and nitrogen, an average of 3 C atoms and 1.1 N atoms per amino acid was assumed.

For determination of D- and L-isomers of the DCAA, hydrolyzed water samples were derivatized with a mixture of OPA and the chiral reagent *N*-acetyl-L-cysteine according to Nimura and Kinoshita (1986). The derivatives were separated by reversed-phase HPLC using the method of Mopper and Furton (1991). Since the applied Nova-Pak C18 column (4.6  $\times$  250 mm, 4  $\mu\text{m}$  particle size; Waters Associates) did not allow a separation of enantiomers of all amino acids

detected by the OPA method, the analytical procedure was optimized for a complete separation of D- and L-enantiomers of Asp, Glu, and Ala. These three amino acids were chosen because they are biologically relevant due to their occurrence in peptidoglycan in bacterial cell walls (Brückner et al. 1994).

## Results

Spring flood carried  $\sim 55$  and 50% of the annual runoff in Lillån and Stridbäcken, respectively. At peak flow, base cations ( $\text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^+ + \text{Na}^+$ ) demonstrated a four- and twofold dilution in Lillån and Stridbäcken, respectively, compared to the winter baseflow (Fig. 1A). In contrast, DCAA increased three- and twofold, respectively (Fig. 1B, Table 2). In both streams, concentrations of DOC and the DOC:DON ratio increased in the beginning of the flood and then decreased during the late flood (Fig. 1B, C). During the peak flood, there was a decrease in DOC-specific light absorbance at 430 nm ( $A_{430}:\text{DOC}$ ) and an increase in the ratio of absorbance at 250 and 365 nm ( $A_{250:365}$ ; Fig. 1C). In both streams, cation dilution and the rise in DCAA and DOC concentrations started around 3 weeks before the apparent flood began (i.e., around the first of April). This indicates a release of water from newly activated soil layers before the initiation of the spring flood (see Discussion).

Concentrations of urea and DFAA varied between 0–2.82 and 0.13–0.90  $\mu\text{M N}$ , respectively, but did not show any clear tendencies (Table 2). Urea and amino acids accounted for 5–18% of DON. DON constituted 90–99.6% of the total dissolved nitrogen in both streams.

The concentration of DON decreased from 36 to 26  $\mu\text{M}$  in early April in Lillån but fluctuated around 30  $\mu\text{M}$  during the rest of the period (Fig. 2A, Table 2). In Stridbäcken, the concentration varied between 17 and 20  $\mu\text{M N}$  during the entire period. During the winter baseflow, the potential bioavailability of DON was 19 and 28% in Lillån and Stridbäcken, respectively. DON bioavailability increased in early April and reached 55 and 45%, respectively, during the peak of the water flow. As the water flow decreased, DON bioavailability also decreased, returning to values similar to the winter baseflow (Stridbäcken) or slightly higher (Lillån).

D-enantiomers constituted 21–38% of Asp, 9–15% of Ala, and 7–16% of Glu in DCAA of the two streams (Fig. 2B). Spring flood influenced the enantiomeric distribution, but in various ways for different amino acids. In Lillån, the proportion of D-enantiomers of all three amino acids was significantly lower during the peak flow than during the winter base flow. However, the proportion of D-Asp had a slight increase in both streams during the early flood. In Stridbäcken, the proportion of D-Glu also peaked in the beginning of the flood. In the latter stream, only D-Ala showed a clear decrease during the flood.

Lillån had a steeper rise in the water flow during the flood than Stridbäcken. The latter stream had several rises and drops in the flow before the maximal flow was reached. Changes in DON bioavailability and in most of the chemical parameters were more pronounced in Lillån than in Stridbäcken. Thus, ratios between the initial and the peak flow

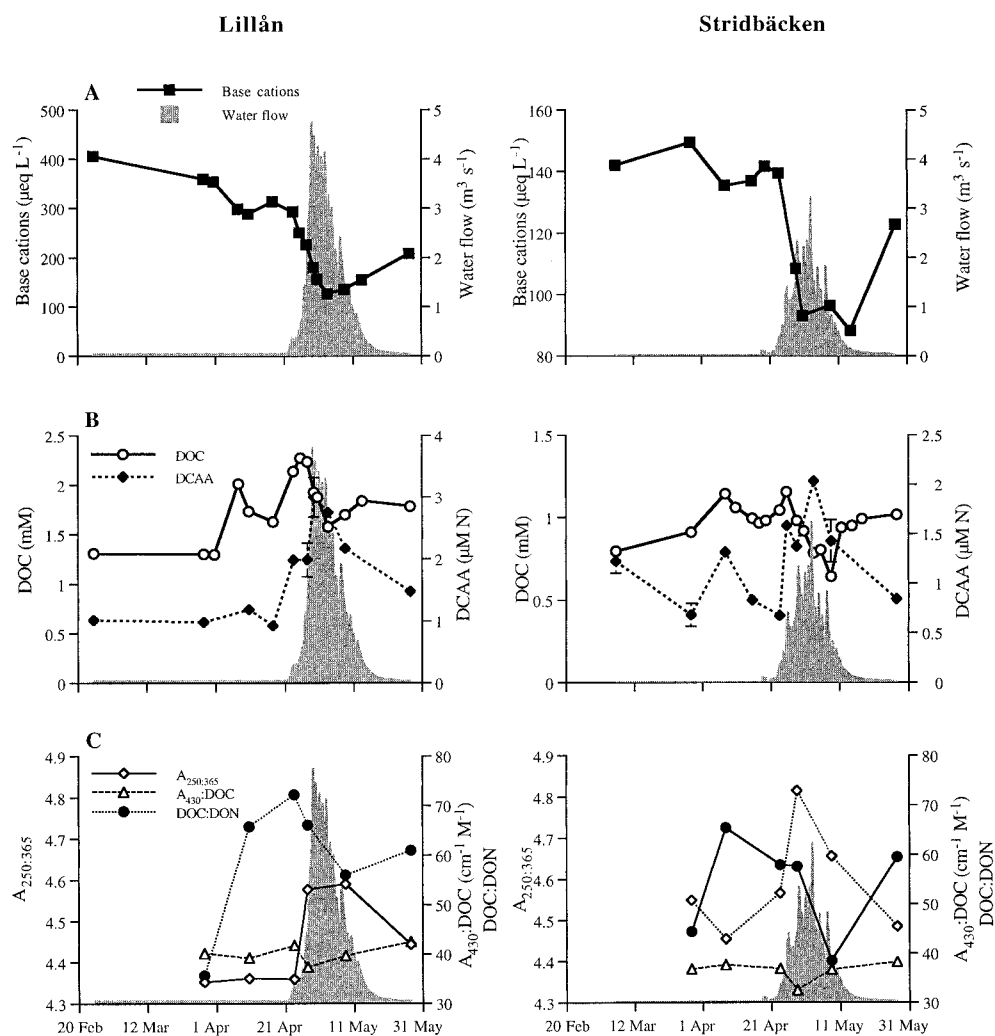


Fig. 1. Chemical and hydrological parameters of Lillån and Stridbäcken streams during the spring flood: (A) water flow and concentrations of base cations; (B) concentrations of DOC and DCAA; (C) ratio of absorbance at 250 and 365 nm ( $A_{250:365}$ ), DOC-specific absorbance at 430 nm ( $A_{430:DOC}$ ), and DOC:DON atom ratio. DCAA values are means  $\pm$  SD ( $n = 3$ ). Other values are from single measurements. In panels B and C, the gray-area graphs represent water flow and show no scale (see panel A).

values of diverse parameters in Lillån and Stridbäcken, respectively, were 2.9 and 1.6 for DON bioavailability, 3.0 and 1.7 for DCAA, 1.8 and 1.5 for DOC, and 0.3 and 0.7 for base cations.

The concentration of potentially bioavailable DON correlated with the concentration of DCAA (Fig. 3A), whereas it did not correlate with concentrations of DFAA, urea, or the sum of all three compound groups. DON bioavailability (the percentage of DON utilized by bacteria) had significant correlations ( $P < 0.05$ ) with water discharge and the fraction of D-Ala (Fig. 3B, Table 3). In both rivers, DON bioavailability tended to correlate positively with the ratio of light absorbance at 250:365 nm, whereas it tended to correlate negatively with the DOC-specific light absorbance at 430 nm (Table 3). Only in Lillån, DON bioavailability tended to have correlations with DOC:DON ratio and the D-enantiomer fraction of aspartate and glutamate.

## Discussion

*Flux of bioavailable DON during spring floods*—The potential bioavailability of DON increased pronouncedly following the spring flood in the two investigated boreal streams (Fig. 2A). The bioavailability increased by a factor of 2.9 in Lillån and 1.6 in Stridbäcken, reaching 55 and 45%, respectively. To our knowledge, no other studies are published on the hydrological control of bulk DON bioavailability. Enhanced access to DOC substrates for bacterioplankton is found during high-water periods in boreal watersheds (Wikner et al. 1999) and in the Amazon River system (Benner et al. 1995). However in these studies, it is not clear if the elevated DOC accessibility is caused by qualitative changes in DOC during the floods or by elevated concentrations of the same quality DOC. In rivers from temperate and subtropical climates, Volk et al. (1997) report no,

Table 2. Water flow and concentrations of dissolved organic nitrogen (DON) compounds in Lillån and Stridbäcken during the spring flood 1998.

| Collection date | Flow (L s <sup>-1</sup> ) | DON compounds (µM N) |       |      |      |      |
|-----------------|---------------------------|----------------------|-------|------|------|------|
|                 |                           | DON                  | PBDON | DCAA | DFAA | Urea |
| Lillån          |                           |                      |       |      |      |      |
| 24 Feb          | 49                        | NA                   | NA    | 1.02 | 0.23 | 0.12 |
| 28 Mar          | 49                        | 36.4                 | 6.8   | 0.99 | 0.24 | 2.82 |
| 10 Apr          | 49                        | 26.3                 | 9.1   | 1.19 | 0.24 | 0.17 |
| 17 Apr          | 49                        | NA                   | NA    | 0.93 | 0.20 | 0.77 |
| 23 Apr          | 339                       | 29.6                 | 11.8  | 1.98 | 0.26 | 0.00 |
| 27 Apr          | 1,494                     | 33.8                 | 18.7  | 1.99 | 0.16 | 0.85 |
| 29 Apr          | 4,214                     | NA                   | NA    | 3.00 | 0.30 | 0.02 |
| 3 May           | 3,071                     | NA                   | NA    | 2.75 | 0.13 | 0.00 |
| 8 May           | 1,405                     | 30.3                 | 12.6  | 2.17 | 0.15 | 0.59 |
| 27 May          | 49                        | 29.2                 | 7.9   | 1.48 | 0.19 | 0.50 |
| Stridbäcken     |                           |                      |       |      |      |      |
| 5 Mar           | 14                        | NA                   | NA    | 1.22 | 0.35 | 2.37 |
| 27 Mar          | 14                        | 20.4                 | 5.7   | 0.68 | 0.20 | 0.44 |
| 6 Apr           | 14                        | 17.4                 | 7.5   | 1.31 | 0.90 | 0.87 |
| 14 Apr          | 14                        | NA                   | NA    | 0.83 | 0.70 | 0.14 |
| 22 Apr          | 346                       | 18.0                 | 8.0   | 0.67 | 0.23 | 0.00 |
| 24 Apr          | 1,193                     | NA                   | NA    | 1.58 | 0.29 | 0.02 |
| 27 Apr          | 1,504                     | 17.0                 | 7.7   | 1.37 | 0.18 | 0.00 |
| 2 May           | 1,486                     | NA                   | NA    | 2.03 | 0.59 | 0.14 |
| 7 May           | 625                       | 16.6                 | 6.9   | 1.43 | 0.19 | 1.03 |
| 26 May          | 20                        | 17.1                 | 5.1   | 0.84 | 0.15 | 0.00 |

NA, not analyzed.

and Leff and Meyer (1991) report a negative, effect of high flows on DOC bioavailability.

Potential DON bioavailability values from the pristine, oligotrophic streams Stridbäcken and Lillån appear to be very high, although only few comparative studies are available. In a similar catchment in South Sweden, only 2–16% of DON was potentially bioavailable during summer and autumn (Stepanauskas et al. 1999a), whereas 40–72% of DON was bioavailable in two large eutrophic rivers in North America (Seitzinger and Sanders 1997). According to a review by Søndergaard and Middelboe (1995), on average 19% of riverine DOC has been found bioavailable.

In boreal regions, spring flood carries up to 50% of the annual runoff within a few weeks (Bishop and Pettersson 1996). Accordingly, Yavitt and Fahey (1986) found that 75% of annual dissolved nitrogen transport from the forest floor occurred during spring snowmelt. DON constituted about 95% of this nitrogen, with high contents of amino acids. In Lillån and Stridbäcken streams, the spring 1998 flood transported 55 and 50% of the annual water discharge, respectively. Because of higher water flow and higher DON bioavailability, the flux (mol h<sup>-1</sup>) of potentially labile DON compounds was higher during the peak flood than during the winter baseflow by two orders of magnitude. Similar patterns probably apply to many northern watersheds. Inorganic nitrogen concentrations in the investigated streams as well as in most pristine watersheds (Hedin et al. 1995) are low. Thus, DON carried by spring floods might constitute more than a half of the total load of potentially bioavailable nitrogen to the boreal coastal waters.

A study by Leff and Meyer (1991) indicated that at high

flow, riverine bacteria are less capable of utilizing dissolved organic material, possibly because bacterial assemblages are dominated by inactive cells washed out from soils. This, together with low water temperatures, might prevent riverine DOM utilization prior to discharge into the sea.

The flood events paralleled a rise in DOC concentrations in Lillån and Stridbäcken, despite the dilution of inorganic solutes (Fig. 1). Accordingly, maximal riverine DOM concentrations generally coincide with periods of elevated surface and groundwater levels associated with snowmelt (Bishop and Pettersson 1996) or increased precipitation (Leff and Meyer 1991). DOC rise during the flood was not accompanied by an increase in bulk DON concentration, resulting in decreased DOC:DON ratio (Figs. 1, 2). However, elevated DON concentrations often occur during snowmelt (Arheimer 1998). Our observed rise in DCAA concentration is in agreement with findings by Volk et al. (1997).

In both streams, changes in the DOM concentrations and character as well as changes in inorganic chemistry seem to precede the observed changes in discharge (Figs. 1, 2). This could be due to changes in the hydrological pathways activating more shallow soil layers already before any noticeable changes in the water level was observed. The quality changes of DOM preceding changes in discharge could also be due to thawing of soil frost releasing previously frozen soil water with a very different chemical composition. Twenty years of soil frost monitoring data from a nearby catchment supports the latter hypothesis since thawing of the soil frost often precedes the spring flood by several weeks (Bishop et al. unpubl. data). In addition, discharge measurements under

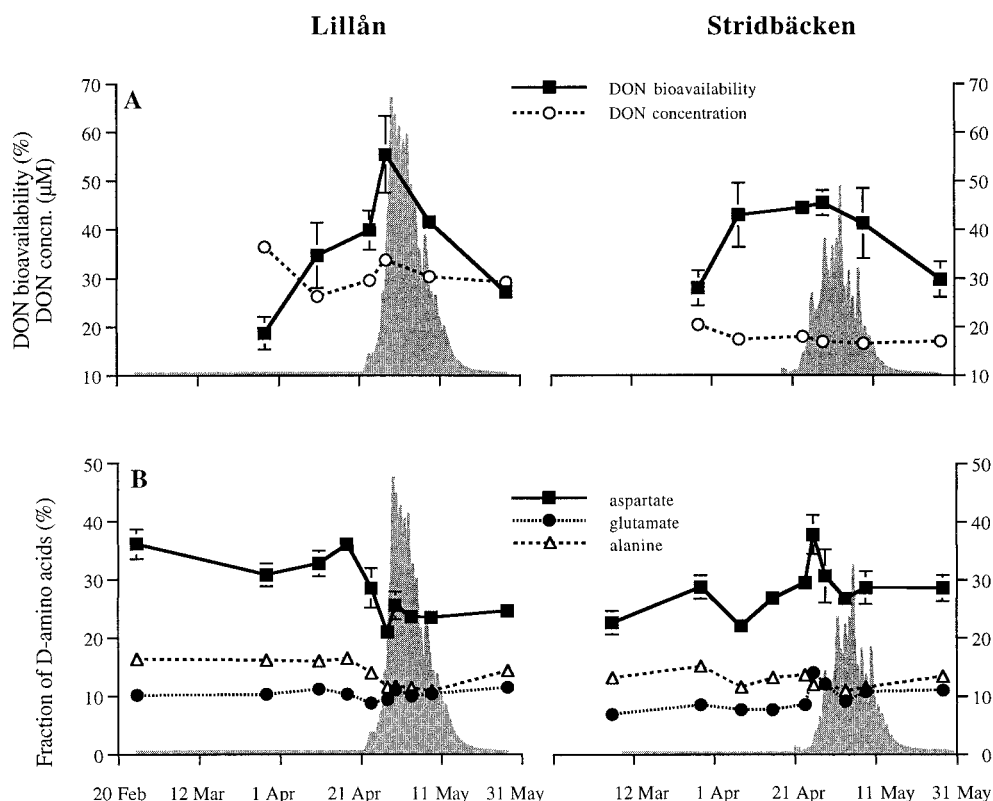


Fig. 2. (A) Dynamics of DON concentration and potential DON bioavailability and (B) DCAA enantiomeric distribution in Lillån and Stridbäcken streams during a spring flood. Values of DON concentration are from single measurements. Other values are means  $\pm$  SD;  $n = 4$  for DON bioavailability and  $n = 3$  for the fraction of D-DCAA. The gray-area graphs represent water flow and show no scale (see Fig. 1A).

the ice cover during the early stages of the flood are technically difficult and may involve flow underestimates.

**Chemical composition of the bioavailable DON**—In our waters, 5–18% of the DON pool was identified as DCAA, DFAA, and urea (Table 2). In general, the percentage of identified DON was low because DCAA alone usually comprised 10–30% of freshwater DON (Jørgensen and Jensen 1997). Assuming all urea, DFAA, and DCAA to be bioavailable, combined they would account for 11–60% of the potentially bioavailable DON (PBDON). Probably, the percentage of identified PBDON was lower since a substantial part of the measurable DCAA is reported to be inaccessible for bacterial uptake (Keil and Kirchman 1994, Tranvik and Jørgensen 1995; Volk et al. 1997; McCarthy et al. 1997). Other N-containing DOM compounds, identifiable with presently available techniques, only account for a marginal part of aquatic DON (Antia et al. 1991).

The low contribution of identified DON compounds to the bacterial N demand in our study suggests that other DON substances were utilized by the bacteria. Most of the dissolved organic matter in fresh waters is identified as humic substances (McKnight and Aiken 1998). However, humic substances include a very broad collection of diverse organic compounds, part of which is biologically available (Tranvik 1998). The majority of DCAA are also found in the humic

fraction (Lytle and Perdue 1981; Volk et al. 1997), and a portion of humic nitrogen might be bound into heterocyclic compounds (McKnight and Aiken 1998; Schulten and Schnitzer 1998).  $^{15}\text{N}$  nuclear magnetic resonance (NMR) analyses of oceanic DON (McCarthy et al. 1997) and soil nitrogen (Schulten and Schnitzer 1998) indicate that the major part of DON occurs in an amide form. Although some amides may originate from amino acids, their abundance suggests that DCAA are not dominant DON components in the ocean and in soils. The amide groups may arise from bacterial cell wall components, which may explain their recalcitrance to microbial degradation (McCarthy et al. 1997, 1998). However, since the amide concentration decreases with depth in the ocean, at least a portion of this nitrogen must be degraded.

A possible source of unidentified DON, including amide groups, might be amino acids associated with organic macromolecules and clays and not detected by the present analytical procedures. Hence, the appearance of vapor phase hydrolysis has increased DCAA recovery up to threefold compared to the traditional liquid phase techniques (Keil and Kirchman 1991; Jørgensen and Jensen 1997). It might be speculated that even the vapor phase hydrolysis is not 100% efficient, resulting in DCAA underestimates. Supporting this hypothesis, Jørgensen et al. (1998) report a 13–23% increase in analytically detectable amino acids after an exposure of

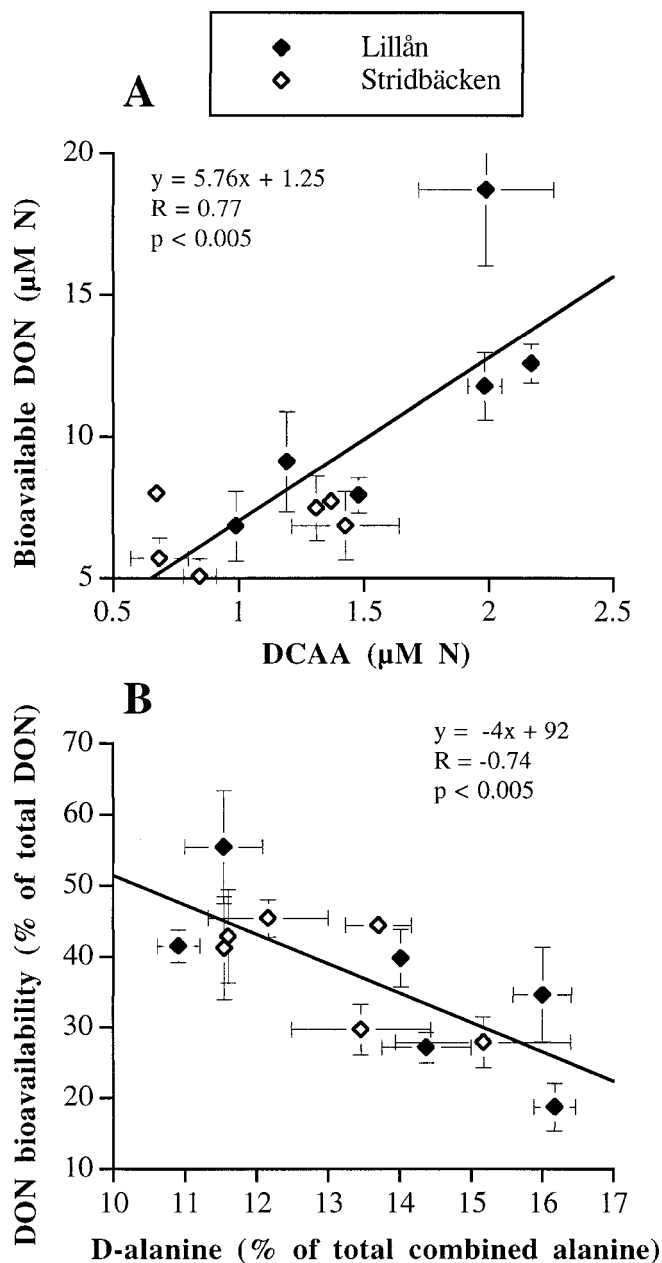


Fig. 3. (A) Correlation between the concentrations of DCAA and the potentially bioavailable DON and (B) correlation between DON bioavailability and the fraction of the D-form of alanine. Values are means  $\pm$  SD;  $n = 4$  for DON bioavailability and  $n = 3$  for DCAA and alanine enantiomeric ratios.

humic water to solar radiation. However, to explain the unidentified PBDON by amino acids in this study, we have to assume that the present analytical techniques underestimated DCAA by a factor of 4 to 11. Intuitively, this does not seem to be realistic.

*Enantiomeric distribution of amino acids*—Chiral properties of amino acids (i.e., whether they are L- or D-isomers) have been suggested to influence their microbial degradation (Pollock et al. 1977; McCarthy et al. 1998; Jørgensen et al.

Table 3. Correlations between the potential DON bioavailability (%), water discharge, and characteristics of DOM;  $n = 6$  for Lillån and Stridbäcken and  $n = 12$  for both rivers together.

|                                  | Lillån | Stridbäcken | Both streams |
|----------------------------------|--------|-------------|--------------|
| Water discharge                  | 0.82*  | 0.60        | 0.71*        |
| DOC : DON                        | 0.71   | -0.03       | 0.42         |
| % D-aspartate                    | -0.68  | -0.13       | -0.48        |
| % D-glutamate                    | -0.53  | -0.01       | -0.23        |
| % D-alanine                      | -0.76  | -0.68       | -0.74**      |
| Light absorbance at 250 :        |        |             |              |
| 365 nm                           | 0.61   | 0.46        | 0.50         |
| Light absorbance at 430 nm : DOC | -0.60  | -0.49       | -0.46        |

\*  $P < 0.05$ ; \*\*  $P < 0.005$ .

1999). The relatively high abundance of D-amino acids (AA) in soils (Pollock et al. 1977) and in the ocean (McCarthy et al. 1998) probably indicates their recalcitrance. However, the recalcitrance of D-amino acids might not be due to the chiral properties per se, since free D-AA are utilized at rates similar to L-AA (O'Dowd and Hopkins 1998), and they are virtually absent in nature (Jørgensen et al. unpubl. data). Probably, the relative recalcitrance of combined D-AA is caused by their presence in structural polymers, such as peptidoglycan in bacterial cell wall, whereas only L-AA occur in proteins. In support of this, Moriarty and Hayward (1982) found a high abundance of bacterial cell wall fragments in marine sediments, suggesting that D-amino acids of prokaryotes contribute significantly to the pool of organic matter in sediments and possibly also in soils.

D-Ala and D-Glu are constitutive components of bacterial cell walls, whereas D-Asp is found in both cell walls and cytoplasm of various organisms (Brückner et al. 1994). Among combined amino acids, D-forms of aspartate, alanine, glutamate, and serine have the highest percentages in diverse environments, as in marine DOM (McCarthy et al. 1998; Jørgensen et al. 1999), riverine DOM (this study), soils (Kimber et al. 1990), and living organisms (Brückner et al. 1994).

In Lillån and Stridbäcken, the proportion of D-isomers of aspartate, glutamate, and alanine showed dynamic changes during the spring flood episode (Fig. 2B). During the peak water flow, the proportion of all D-enantiomers decreased in Lillån. In Stridbäcken, only the percentage of D-Ala showed a clear decrease during the flood. In both streams, aspartate had the highest percentage of D-isomers among the three amino acids. There was a great variation in the occurrence of D-aspartate in Lillån and Stridbäcken streams, as well as in environments investigated by other authors (Fig. 4). However, our studied streams tended to have higher values than the marine sites. An exception was the Gulf of Riga, which is a brackish water body with extensive riverine inputs of organic material. The frequencies of D-Ala similar to the ones in Lillån and Stridbäcken are found in soils (Kimber et al. 1990) and bacterial biomass from various pelagic environments (Jørgensen et al. unpubl. data). On the contrary, D-Ala frequencies, two to three times higher than our measurements, were found by McCarthy et al. (1998) in high

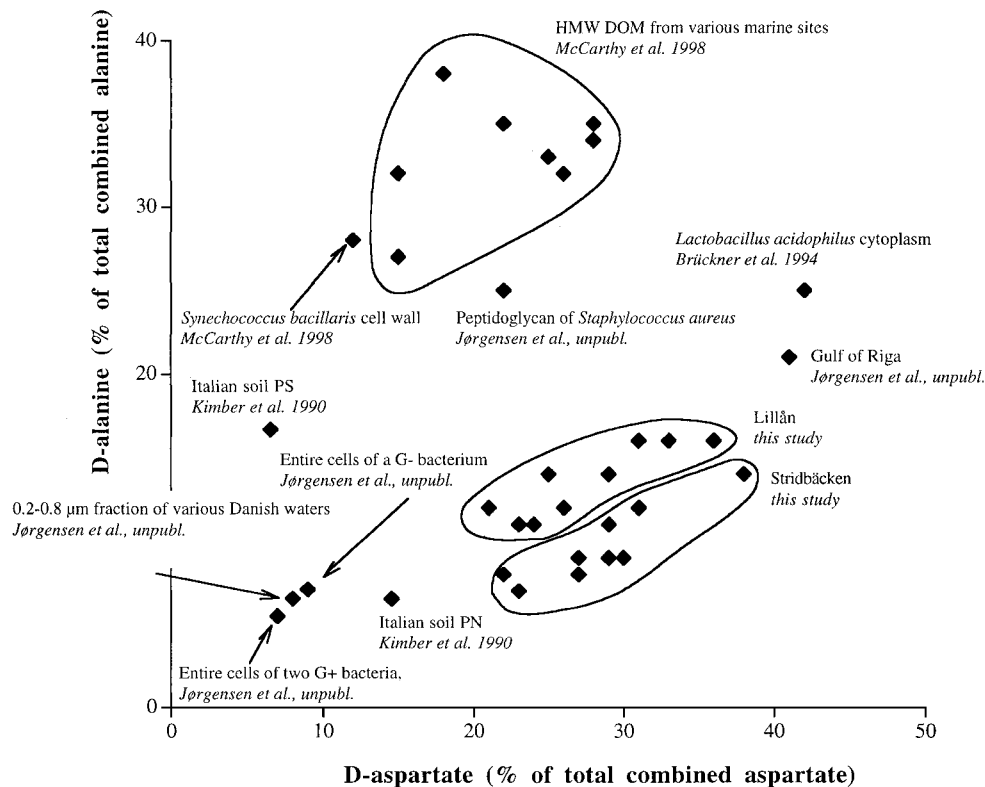


Fig. 4. Proportion of D-enantiomers of aspartate and alanine in various environments.

molecular weight (HMW) DOM collected from a range of marine sites.

The comparison of various environments reveals that enantiomeric ratios of different amino acids (or at least alanine and aspartate) do not correlate with each other (Fig. 4). Obviously, contrasting sources or sinks of separate amino acid enantiomers, or both, exist, but too little is known to explain the observed patterns. For example, it is difficult to explain why Lillån and Stridbäcken were separated in the D-Asp: Ala scattergram (Fig. 4;  $P < 0.001$ , analysis of covariance) since both streams are similar and geographically close. Possibly, enantiomeric analyses of amino acids may be used to trace DON sources.

The correlation between the DON bioavailability and the percentage D-Ala (Fig. 3) indicates that the enantiomer ratio of amino acids may be used as an indicator of DON bioavailability. Similar correlations between DCAA enantiomeric ratios and DON degradability have been reported by Jørgensen et al. (1999) from the Gulf of Riga. The advantage of the enantiomer analysis is that the bioavailability can be examined in preserved samples and that no incubation is required.

*Sources of bioavailable DON*—During baseflow conditions in forested areas, runoff mainly originates from deeper mineral soil horizons and the groundwater zone. During spring floods, infiltration of snowmelt water into the soil raises the water table, which activates water flow through more superficial soil horizons rich in DOM (Bishop and Pettersson 1996). The situation is different in most boreal wet-

lands, where water runoff is mainly derived from superficial horizons during the entire year. Thus, in wetland-rich catchments, fewer new DOM sources are released during the snowmelt compared to mainly forested catchments. Eckhardt and Moore (1990) found a relationship between DOM concentration and flow fluctuations only in catchments with a low share of wetlands. A lower proportion of wetlands in the Lillån watershed compared to Stridbäcken (Table 1) might explain the higher per-area transport of DON and DOC and the larger fluctuations in DOM character in the former stream during the flood (Figs. 1, 2). In addition, new DOM sources might be depleted gradually during numerous rises and falls of the water table before the peak flood in Stridbäcken.

High DON bioavailability, increases in DCAA, and decreases in the proportion of D-amino acids (Figs. 1, 2) indicated that a large quantity of relatively fresh material was transported away from the catchment during the spring flood. Accordingly, decreased DOC-specific absorbance ( $A_{430} : \text{DOC}$ ) and increased absorbance ratio ( $A_{250:365}$ ) indicated that flood DOM was less lignified and had lower average molecular weight than baseflow DOM (de Haan 1972; Pages and Gadel 1990; Stepanauskas et al. 1999a,b), despite the elevated DOC:DON ratio (Fig. 1C).

Flushing of relatively fresh detritus from surface soil horizons by rising groundwater could be one explanation for the improved DOM quality in streams during the spring flood. In addition, Edwards et al. (1986) report physicochemical mobilization of particulate detritus into solution during soil freezing. Finally, several authors suggest that the

disruption of microbial cells during freeze–thaw events contributes significantly to the concentration of DON in soil solution (Christensen and Christensen 1991; Deluca et al. 1992). Microbial (bacterial and fungal) nitrogen constitutes around 30 kg ha<sup>-1</sup>, or 10%, of total soil nitrogen in northern Swedish forests and may be an order of magnitude higher in wetland soils (Näsholm et al. 1998). Roughly, around 1 and 0.5 kg DON ha<sup>-1</sup> were flushed from Lillån and Stridbäcken watersheds during the spring flood (assuming 220 and 200 mm specific runoff and 31 and 18 μM DON concentrations, respectively). Thus, a lysis of not more than 2–3% of soil microbial cells could support the entire DON washout during the flood.

## References

- ANTIA, N. J., P. J. HARRISON, AND L. OLIVEIRA. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology, and ecology. *Phycologia* **30**: 1–89.
- ARHEIMER, B. 1998. Riverine nitrogen—analysis and modelling under Nordic conditions. Ph.D. thesis, Linköping Univ., Sweden.
- BENNER, R., S. OPSAHL, G. CHIN-LEO, J. E. RICHEY, AND B. R. FORSBERG. 1995. Bacterial carbon metabolism in the Amazon River system. *Limnol. Oceanogr.* **40**: 1262–1270.
- BERTILSSON, S., R. STEPANAUSKAS, R. CUADROS-HANSSON, W. GRANÉLI, J. WIKNER, AND L. TRANVIK. 1999. Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. *Aquat. Microb. Ecol.* **19**: 47–56.
- BISHOP, K., AND C. PETTERSSON. 1996. Organic carbon in the boreal spring flood from adjacent subcatchments. *Environ. Int.* **22**: 535–540.
- BRÜCKNER, H., S. HAASMANN, M. LANGER, T. WESTHAUSER, AND R. WITTNER. 1994. Liquid chromatographic determination of D- and L-amino acids by derivatization with *o*-phthalaldehyde and chiral thiols. *J. Chromatogr.* **666**: 259–273.
- CHANEY, A., AND E. P. MARBACH. 1962. Modified reagents for determination of urea and ammonia. *Clin. Chem.* **8**: 130–132.
- CHRISTENSEN, S., AND B. T. CHRISTENSEN. 1991. Organic matter available for denitrification in different soil fractions: Effect of freeze/thaw cycles and straw disposal. *J. Soil Sci.* **42**: 637–647.
- DE HAAN, H. 1972. Molecule-size distribution of soluble humic compounds from different natural waters. *Freshw. Biol.* **2**: 235–241.
- DEL GIORGIO, P. A., D. F. BIRD, Y. T. PRAIRIE, AND D. PLANAS. 1996. The flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain SYTO 13. *Limnol. Oceanogr.* **41**: 783–789.
- DELUCA, T. H., D. R. KEENEY, AND G. W. MCCARTY. 1992. Effect of freeze–thaw events on mineralization of soil nitrogen. *Biol. Fertil. Soils* **14**: 116–120.
- ECKHARDT, B. W., AND T. R. MOORE. 1990. Controls on dissolved organic carbon concentrations in streams, southern Québec. *Can. J. Fish. Aquat. Sci.* **47**: 1537–1544.
- EDWARDS, A. C., J. CREASEY, AND M. S. CRESSER. 1986. Soil freezing effects on upland stream solute chemistry. *Water Res.* **20**: 831–834.
- GUILLARD, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. *In* W. L. Smith and M. H. Chamby [eds.], *Culture of marine invertebrate animals*. Plenum Press.
- HEDIN, L. O., J. J. ARMESTO, AND A. H. JOHNSON. 1995. Patterns of nutrient loss from unpolluted, old-growth temperate forests: evaluation of biogeochemical theory. *Ecology* **76**: 493–509.
- JØRGENSEN, N. O. G., AND R. E. JENSEN. 1997. Determination of dissolved combined amino acids using microwave-assisted hydrolysis and HPLC precolumn derivatization for labeling of primary and secondary amines. *Mar. Chem.* **57**: 287–297.
- , N. KROER, R. C. COFFIN, X.-H. YANG, AND C. LEE. 1993. Dissolved free amino acids, combined amino acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Mar. Ecol. Prog. Ser.* **98**: 135–148.
- , L. TRANVIK, H. EDLING, W. GRANÉLI, AND M. LINDELL. 1998. Effects of sunlight on occurrence and bacterial turnover of specific carbon and nitrogen compounds in lake water. *FEMS Microb. Ecol.* **25**: 217–227.
- , ———, AND G. M. BERG. 1999. Occurrence and bacterial cycling of dissolved nitrogen in the Gulf of Riga, the Baltic Sea. *Mar. Ecol. Prog. Ser.* **191**: 1–18.
- KEIL, R. G., AND D. L. KIRCHMAN. 1991. Dissolved combined amino acids in marine waters as determined by a vapor-phase hydrolysis method. *Mar. Chem.* **33**: 243–259.
- , AND ———. 1994. Abiotic transformation of labile protein to refractory protein in sea water. *Mar. Chem.* **45**: 187–196.
- KIMBER, R. W., P. NANNIPIERI, AND B. CECCANTI. 1990. The degree of racemization of amino acids released by hydrolysis of humic-protein complexes: Implications for age assessment. *Soil Biol. Biochem.* **22**: 181–185.
- LAUDON, H. 1999. Spring flood pH decline in northern Sweden: Towards an operational model for separating natural acidity from anthropogenic acidification. Licentiate thesis. Swedish Univ. of Agricultural Sciences, Sweden.
- LEFF, L. G., AND J. L. MEYER. 1991. Biological availability of dissolved organic carbon along the river. *Limnol. Oceanogr.* **36**: 315–323.
- LINDROTH, P., AND K. MOPPER. 1979. High performance liquid chromatographic determinations of subpicomole amounts of amino acids by precolumn fluorescence derivatization with *o*-phthalaldehyde. *Anal. Chem.* **51**: 1667–1674.
- LYTLE, C. R., AND E. M. PERDUE. 1981. Free, proteinaceous, and humic-bound amino acids in river water containing high concentrations of aquatic humus. *Environ. Sci. Technol.* **15**: 224–228.
- MCCARTHY, M. D., T. PRATUM, J. I. HEDGES, AND R. BENNER. 1997. Chemical composition of dissolved organic nitrogen in the ocean. *Nature* **390**: 150–154.
- , J. I. HEDGES, AND R. BENNER. 1998. Major bacterial contribution to marine dissolved organic nitrogen. *Science* **281**: 231–234.
- McKNIGHT, D. M., AND G. R. AIKEN. 1998. Sources and age of aquatic humus, p. 9–40. *In* D. O. Hessen and L. J. Tranvik [eds.], *Aquatic humic substances*. Springer-Verlag.
- MOPPER, K., AND K. G. FURTON. 1991. Extraction and analysis of polysaccharides, chiral amino acids, and SFE-extractable lipids from marine POM. *Geophys. Monogr.* **63**: 151–161.
- MORIARTY, D. J. W., AND A. C. HAYWARD. 1982. Ultrastructure of bacteria and the proportion of gram-negative bacteria in marine sediments. *Microb. Ecol.* **8**: 1–14.
- NÄSHOLM, T., A. EKBLAD, A. NORDIN, R. GIESLER, M. HÖGBERG, AND P. HÖGBERG. 1998. Boreal forest plants take up organic nitrogen. *Nature* **392**: 914–916.
- NIMURA, N., AND T. KINOSHITA. 1986. *o*-Phthalaldehyde-*N*-acetyl-L-cysteine as a chiral derivatization agent for liquid chromatographic optical resolution of amino acid enantiomers and its application to conventional amino acid analysis. *J. Chromatogr.* **352**: 169–177.
- O'DOWD, R. W., AND D. W. HOPKINS. 1998. Mineralization of carbon from D- and L-amino acids and D-glucose in two contrasting soils. *Soil Biol. Biochem.* **30**: 2009–2016.
- PAGES, J., AND F. GADEL. 1990. Dissolved organic matter and UV

- absorption in a tropical hyperhaline estuary. *Sci. Total Environ.* **99**: 173–204.
- POLLOCK, G. E., C. N. CHENG, AND S. E. CRONIN. 1977. Determination of the D and L isomers of some protein amino acids present in soils. *Analyt. Chem.* **49**: 2–7.
- PRICE, N. M., AND P. J. HARRISON. 1987. Comparison of methods for the analysis of dissolved urea in seawater. *Mar. Biol.* **94**: 307–317.
- RYTHER, J. H., AND W. M. DUNSTAN. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* **171**: 1008–1013.
- SCHULTEN, H. R., AND M. SCHNITZER. 1998. The chemistry of soil organic nitrogen: A review. *Biol. Fertil. Soils* **26**: 1–15.
- SEITZINGER, S. P., AND J. G. SANDERS. 1997. Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Mar. Ecol. Prog. Ser.* **159**: 1–12.
- SØNDERGAARD, M., AND M. MIDDLEBOE. 1995. A cross-system analysis of labile dissolved organic carbon. *Mar. Ecol. Prog. Ser.* **118**: 283–294.
- STEPANAUSKAS, R., L. J. TRANVIK, AND L. LEONARDSON. 1999a. Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton. *Limnol. Oceanogr.* **44**: 1477–1485.
- , H. EDLING, AND L. J. TRANVIK. 1999b. Differential dissolved organic nitrogen availability and bacterial aminopeptidase activity of limnic and marine waters. *Microb. Ecol.* **38**: 264–272.
- TRANVIK, L. J. 1998. Degradation of dissolved organic matter in humic waters by bacteria, p. 259–284. *In* D. O. Hessen and L. J. Tranvik [eds.], *Aquatic humic substances*. Springer-Verlag.
- , AND N. O. G. JØRGENSEN. 1995. Colloidal and dissolved organic matter in lake water: Carbohydrate and amino acid composition, and ability to support bacterial growth. *Biogeochem. Dordr.* **30**: 77–97.
- VOLK, C. J., C. B. VOLK, AND L. A. KAPLAN. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. *Limnol. Oceanogr.* **42**: 39–44.
- WIKNER, J., R. CUADROS, AND M. JANSSON. 1999. Differences in consumption of allochthonous DOC under limnic and estuarine conditions in a watershed. *Aquat. Microb. Ecol.* **17**: 289–299.
- WOOD, E. D., F. A. J. ARMSTRONG, AND F. A. RICHARDS. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *J. Mar. Biol. Assoc. U.K.* **47**: 23–31.
- YAVITT, J. B., AND T. J. FAHEY. 1986. Litter decay and leaching from the forest floor in *Pinus contorta* (lodgepole pine) ecosystems. *J. Ecol.* **74**: 525–545.

Received: 15 December 1999

Accepted: 7 June 2000

Amended: 28 June 2000