

Mangrove tannins in aquatic ecosystems: Their fate and possible influence on dissolved organic carbon and nitrogen cycling

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Abstract

We describe the fate of mangrove leaf tannins in aquatic ecosystems and their possible influence on dissolved organic nitrogen (DON) cycling. Tannins were extracted and purified from senescent yellow leaves of the red mangrove (*Rhizophora mangle*) and used for a series of model experiments to investigate their physical and chemical reactivity in natural environments. Physical processes investigated included aggregation, adsorption to organic matter-rich sediments, and co-aggregation with DON in natural waters. Chemical reactions included structural change, which was determined by excitation–emission matrix fluorescence spectra, and the release of proteins from tannin–protein complexes under solar-simulated light exposure. A large portion of tannins can be physically eliminated from aquatic environments by precipitation in saline water and also by binding to sediments. A portion of DON in natural water can coprecipitate with tannins, indicating that mangrove swamps can influence DON cycling in estuarine environments. The chemical reactivity of tannins in natural waters was also very high, with a half-life of less than 1 d. Proteins were released gradually from tannin–protein complexes incubated under light conditions but not under dark conditions, indicating a potentially buffering role of tannin–protein complexes on DON recycling in mangrove estuaries. Although tannins are not detected at a significant level in natural waters, they play an important ecological role by preserving nitrogen and buffering its cycling in estuarine ecosystems through the prevention of rapid DON export/loss from mangrove fringe areas and/or from rapid microbial mineralization.

Mangrove forests represent an important ecosystem in tropical and subtropical coastal fringes and cover approximately 1.7×10^5 km² worldwide (Valiela et al. 2001). The biogeochemical, ecological, and economical importance of the mangrove ecosystem has been recognized recently (Valiela et al. 2001). For example, mangroves play an important role in global carbon cycling by acting as a sink of CO₂ (Ong 1993) and also as a significant source of dissolved organic matter (DOM) to the world oceans (Dittmar et al. 2006). While both aboveground and belowground productivity in mangrove forests contribute to the high organic matter content in mangrove soils (Comley and McGuinness 2005), litterfall plays a crucial role in the nutrient cycling of mangrove forests (Odum and Heald 1975; Twilley et al. 1986). Indeed, litterfall, which

consists mainly of leaves, represents about one third of primary production in mangrove forests (Robertson et al. 1992). Under submerged conditions the abscised leaves release substantial amounts of DOM containing sugars, proteins, and polyphenols, as well as inorganic nutrients, to the surrounding aquatic environment within a relatively short time period (Benner et al. 1990; Maie et al. 2006a). Sugars and proteins are generally believed to be susceptible to microbial degradation and thus can be quickly incorporated into food webs (Gremm and Kaplan 1998; Weiss and Simon 1999). However, tannins, a class of polyphenols, are known to suppress microbial activity (Kuiters 1990; Hättenschwiler and Vitousek 2000; Kraus et al. 2003), and as such can affect biogeochemical cycling in these ecosystems.

Tannins are major constituents of vascular plants, along with cellulose, hemicellulose, and lignins (De Leeuw and Largeau 1993), and sometimes comprise more than 20% of the dry weight of plant materials. Their concentration varies among vegetation types (Hernes and Hedges 2004), organs (Preston 1999), growing stages (Lin et al. 2006), and environmental conditions (Northup et al. 1998). The concentration of tannins can exceed that of lignins in soft tissues, such as foliage, flowers, and fine roots (Kraus et al. 2003).

Tannin polymers are mostly water-soluble and highly reactive, and their ecological effect in soil sciences has been widely investigated (Kuiters 1990; Kraus et al. 2003). Among these ecological effects is their protein-binding ability, influencing nitrogen (N) dynamics in ecosystems (Northup et al. 1998; Schimel et al. 1998; Preston 1999). Tannins form microbially recalcitrant complexes with proteins; they also deactivate exoenzymes, preventing them

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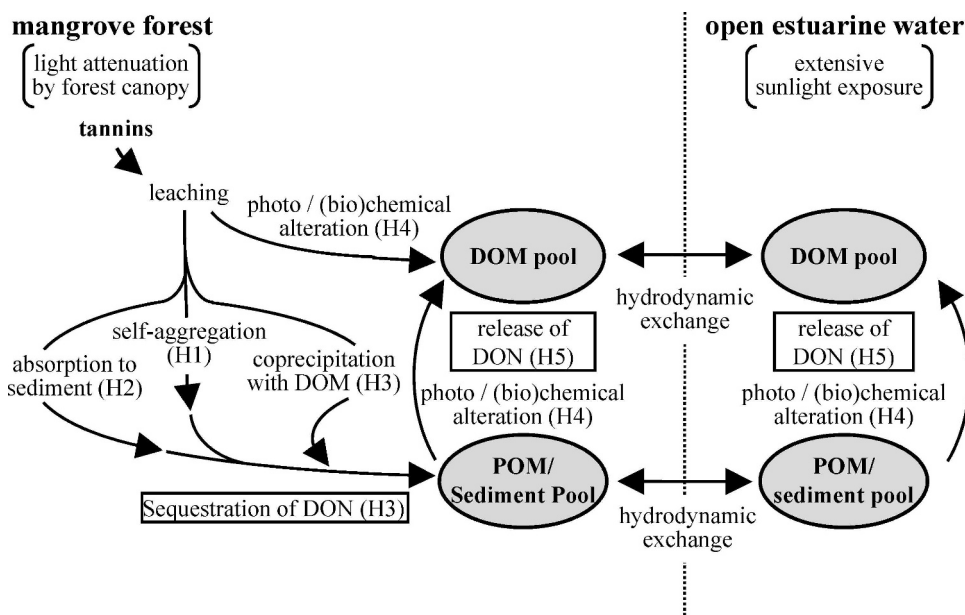


Fig. 1. Conceptual diagram of dynamics of tannins in aquatic environments. The hypothesis of each process, denoted in parentheses, was examined in the corresponding section.

from rapid microbial mineralization (Baldwin et al. 1983). As a result, their presence can affect the efficiency and the rate of nutrient cycling (Schimel et al. 1998; Bradley et al. 2000).

Although the ecological importance of tannins has been widely recognized in the field of soil science and plant nutrition, there is no information on their fate in aquatic environments. DOM freshly leached from wetland plant biomass contains significant amounts of dissolved organic nitrogen (DON) (Benner et al. 1990; Davis et al. 2003), which is thought to play a significant role in biogeochemical processes in aquatic ecosystems. Since mangroves, especially those involving the Rhizophoraceae family, contain a large amount of tannins (Basak et al. 1999; Hernes and Hedges 2004), it is expected that tannins leached from mangrove leaves may sequester proteins in aquatic ecosystems. Previous studies from our laboratory indicate that (1) mangrove (*Rhizophora mangle*) leachate contains a large amount of polyphenols (Maie et al. 2006a); (2) tannin concentrations are quite low in natural waters, even in areas surrounded by mangrove forests (Maie et al. 2006a); and (3) leachates from mangrove leaves are photosensitive (Scully et al. 2004). This information indicates that mangrove tannins are likely highly reactive and may undergo both physical and chemical transformations after their release into natural waters. These processes may affect N cycling through the formation of stable tannin–protein complexes. To address this hypothesis, we proposed a conceptual model for the biogeochemical processing of mangrove tannins in coastal ecosystems (Fig. 1). Each process depicted in the figure was examined by a series of simple model experiments, as described below.

Aggregation of tannins (H1)

Tannin molecules have vicinal hydroxyl groups, which can act as chelates with metals (Kraus et al. 2003). Since

natural waters, especially saline waters, contain a high concentration of metals and salts, tannins may be precipitated out by aggregation (intermolecular associations) through interactions with polyvalent cations in coastal and estuarine zones. In this experiment, we investigate the aggregation/precipitation behavior of tannins along a salinity gradient.

Sorption of tannins to sediments (H2)

Tannins have a strong affinity to soils (Schofield et al. 1998; Bradley et al. 2000; Kaal et al. 2005). As such, tannins in natural waters may be sequestered by sorption on suspended solids and/or underlying peat soils, even under flooded conditions. In this experiment, sorption of tannins on a mangrove peat soil was examined.

Sequestration of DON in natural waters by tannins (H3)

Tannins form insoluble complexes with proteins, which are resistant to microbial degradation (reviewed by Kraus et al. 2003). Although no such studies have been conducted in aquatic ecosystems, tannins could potentially sequester protein-like DON in mangrove estuaries and significantly reduce protein export into coastal regions. In this experiment, we examined the formation of insoluble complexes between tannins and DON in natural waters.

Alteration of tannin structure (H4)

Tannins are chemically reactive (Kraus et al. 2003; Maie et al. 2003; Nierop et al. 2006); as such, tannins might transform quickly in aqueous environments. Furthermore, since tannins have strong light absorptive properties, photochemical reactivity is expected (Forest et al. 2004).

Table 1. Characteristics of natural water samples collected from the Florida Coastal Everglades.*

Location	Ecosystem	DOC (mg C L ⁻¹)	DON (mg N L ⁻¹)	TP (μg P L ⁻¹)	Salinity	Dominant vegetation	Location
T2	Freshwater marsh	6.8	0.45	3.1	0	Sawgrass (<i>Cladium jamaicense</i>); spikerush (<i>Eleocharis</i> spp.); periphyton	25°24'N, 80°36'W
T6	Mangrove swamp	7.7	0.46	3.7	0.3	Mangrove (<i>Rhizophora mangle</i>)	25°13'N, 80°39'W
T10	Estuarine	7.9	0.61	8.1	32	Seagrass; turtle grass (<i>Thalassia testudinum</i>)	25°01'N, 80°41'W

* DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TP, total phosphorus.

This experiment focused on the structural alteration of tannins in aquatic environments and the influence of light on its reactivity.

Dissociation of tannin–protein complexes (H5)

Tannin–protein complexes have been shown to be recalcitrant to microbial degradation (Kraus et al. 2003). However, Maie et al. (2003) showed that the molecular structure of tannins was modified during degradation of foliage, accompanied by a decrease in the protein binding capacity. For this reason, it could be expected that biolabile proteins can be re-released into the water column through the diagenetic alteration of insoluble tannin–protein complexes. This experiment focused on the breakdown of tannin–protein complexes during (photo)chemical alteration.

Materials and methods

Isolation and characterization of tannins—Tannins were extracted and purified from nearly senescent, yellow-colored leaves of red mangroves (*Rhizophora mangle*), according to the method of Maie et al. (2003). Briefly, freeze-dried mangrove leaves were soxhlet-extracted with hexane. The tannins were isolated from the solid residue by an acetone/water solution (70:30), to which 0.1% (w/v) ascorbic acid was added. The crude extract was first cleaned by several washings with dichloromethane and ethyl acetate and was then purified by column chromatography on Sephadex™ LH-20 (Amersham Pharmacia Biotech AB). The purified tannins were freeze-dried and powdered. A tannin stock solution (2,000 mg L⁻¹) was prepared fresh on the day of the experiment by dissolving powder tannins in Milli-Q® (Millipore) water. Tannin solutions with concentrations of 20 mg L⁻¹ or 10.6 mg carbon (C) L⁻¹ were used in most of the experiments. This concentration may be somewhat higher than that in the natural environment but was intended to facilitate the detection of tannins.

The purity and chemical characteristics of the tannins were confirmed by solution ¹³C nuclear magnetic resonance (NMR) spectroscopy. The NMR spectrum was obtained at 100.62 MHz on a Bruker AM400 spectrometer using a 5-mm probe. Purified tannin powder was dissolved in a D₂O/

acetone-d₆ (1:1) mixture. A standard pulse sequence with inverse gated decoupling using a 30° excitation pulse was applied to obtain a quantitative spectrum (Maie et al. 2003).

Natural water and peat samples—Coastal wetland surface-water samples were collected from study sites established by the Florida Coastal Everglades Long Term Ecological Research (FCE-LTER) program (<http://fcelter.fiu.edu/>) (Table 1). A low dissolved organic carbon (DOC)–concentration marine water sample was collected from surface Gulf Stream water flowing offshore of the Florida Keys. Natural water samples were filtered through GF/F glass-fiber filters and stored at 4°C until use. A mangrove peat soil sample was collected from site SRS-4 (25°41'N, 80°96'W), one of the FCE-LTER stations, where the dominant vegetation is mangrove (mixture of *R. mangle*, *Conocarpus erectus*, and *Laguncularia racemosa*). The soil sample was wet-sieved through a 0.5-mm sieve to remove large particles.

Filtration of water samples—Precombusted (450°C for 4 h) GF/F glass-fiber filters (nominal pore size, 0.7 μm; Whatman International Ltd.) were used for the filtration of water samples. Although the pore size of the filter was not small enough to entirely eliminate all bacteria, the filter was chosen to avoid DOC contamination from the filter and adsorption of tannins onto the filter.

DOC and total dissolved N (TDN) analysis—DOC concentrations were analyzed using a high-temperature catalytic combustion method on a Shimadzu TOC-V total organic carbon (TOC) analyzer. Samples were acidified (pH < 2) with concentrated HCl in a built-in syringe of the TOC analyzer and were purged for 5 min with N₂ to remove inorganic C just before the injection. The practical quantitation limit (PQL) of the measurement was 0.27 mg L⁻¹. TDN was measured on an ANTEK 9000 Nitrogen Analyzer using a previously reported modification to the standard method (Frankovich and Jones 1998). The PQL of the measurement was 0.04 mg L⁻¹.

Colorimetric analysis of total phenols—Total phenol concentration, a proxy for tannin concentration, was analyzed using the Folin–Ciocalteu method (Waterman and Mole 1994). A 0.2–5-mL aliquot was pipetted into a test tube and the volume was adjusted to 5 mL with Milli-Q water.

Then a 250- μL aliquot of Folin–Ciocalteu reagent (Sigma-Aldrich) was added and vortexed. After 5 min, 750 μL of 20% (w/v) calcium carbonate solution was added and vortexed. After standing for 2 h at room temperature, the absorbance at 760 nm was measured on a Cary 50 ultraviolet (UV)-visible (Vis) spectrophotometer (Varian). Solutions of purified mangrove tannins, with concentrations ranging from 1 to 10 mg L^{-1} , were used as calibration standards.

Colorimetric analysis of total hydrolyzable amino acid (THAA)—Dissolved protein concentration was measured colorimetrically after hydrolysis as THAA concentration, according to the method of Castell et al. (1979). A 2-mL aliquot of water sample, 20 μL of 11 mmol L^{-1} ascorbic acid, and 2 mL of 12 mol L^{-1} HCl were pipetted into a 15-mL heavy-wall pressure vessel (Chemglass). After flushing with argon, the vessel was closed tight with a Teflon bushing and heated at 110°C for 24 h in an oven. The hydrolysate was cooled and neutralized with 10 mol L^{-1} NaOH. A 100- μL aliquot of hydrolysate, 100 μL of borate buffer, and 100 μL of fluorescamine were pipetted into a fluorescence cuvette and allowed to react for 1 min. The reaction was terminated by adding 2.5 mL of Milli-Q water. The fluorescence emission at 475 nm at an excitation of 390 nm was measured on a Fluoromax-3 spectrofluorometer (Horiba Jobin Yvon). Glycine solutions, with concentrations ranging from 0.1 to 0.8 mg L^{-1} , were used as calibration standards.

UV-Visible absorption spectroscopy—The slope of the UV-Visible absorption spectra of DOM is often used to compare the degree of the development of the electron transfer system of humic substances (e.g., humic substances with a lower slope have a more developed electron transfer system) (Kumada 1987). UV-visible absorption spectra were determined on a Cary 50 UV-Vis spectrophotometer, and the ratio of the absorption at 300 nm to 350 nm (A_{300}/A_{350}) was calculated as an index of the slope. Milli-Q water was measured as the blank and was subtracted from the sample spectra.

Fluorescence spectroscopy—Excitation–emission matrix (EEM) fluorescence spectra were measured as a proxy for tannin concentration and to provide insight regarding changes in the tannin structure. Forty emission scans were acquired at excitation wavelengths (λ_{ex}) between 260 and 455 nm at 5-nm intervals on a Fluoromax-3 spectrofluorometer according to the method of Maie et al. (2006b). The emission wavelengths (λ_{em}) were scanned from $\lambda_{\text{ex}} + 10$ nm to $\lambda_{\text{ex}} + 250$ nm at 2-nm intervals. The individual spectra were concatenated to form EEM. All fluorescence spectra were acquired in ratio mode. Inner filter effects were corrected according to the method of McKnight et al. (2001). Instrument biases related to wavelength-dependent efficiencies of the specific instrument's optical components were corrected by applying multiplication factors supplied by the manufacturer. The fluorescence intensity values were converted to quinine sulfate units. Milli-Q water was measured as the blank and subtracted from the sample spectra. Tannin concentrations were estimated from the

emission peak intensity at a $\lambda_{\text{ex}}/\lambda_{\text{em}}$ value of 280/314 nm. The standard error was less than 0.5%.

Self-aggregation of tannins (H1)

Two milliliters of tannin stock solution (ca. 2,000 mg L^{-1}) was mixed with 200 mL of artificial seawater (Instant Ocean®, Synthetic sea salt, Aquarium Systems) at different salinities (0, 5, 10, 15, 25, and 35 mg L^{-1}) in 250-mL Nalgene® brown bottles (Nalge Nunc International) and shaken gently on a reciprocal shaker for 24 h. After filtration, the DOC concentration in the solution, which originated from tannins, was measured as a proxy for tannin concentration. The blank samples were prepared in the same manner, but by mixing Milli-Q water instead of tannin stock solution, and were used for correction. The incubations were conducted in duplicate.

Adsorption of tannins to peat soil (H2)

A 10-mL sample of peat soil slurry (1.00 g dry weight) was mixed with 100 mL of tannin solution of various concentrations (0, 5, 10, 20, 40, and 80 mg L^{-1}) and gently shaken on a reciprocal shaker for 6 h. The suspension was centrifuged (1,100 \times g for 20 min) and filtered, and the DOC and total phenol concentrations of the supernatant were analyzed. The rate of tannin adsorption on peat soil was investigated with two solutions of different tannin concentrations (20 and 80 mg L^{-1}) and by varying the reaction time (0.5, 1, 2, 4, 6, and 12 h). The incubations were conducted in duplicate.

Sequestration of DON in natural water by tannins (H3)

Various amounts of tannin stock solution (2,000 mg L^{-1}) were mixed with 150 mL of natural-water samples of known DOC and TDN concentrations collected from sites T2, T6, and T10 to achieve final concentrations of 0, 5, 10, 20, 40, and 80 mg L^{-1} in 250-mL Nalgene brown bottles. The bottles were allowed to stand for 24 h at room temperature (23°C). The solutions were filtered and the DOC and TDN concentrations were determined. Since tannins used in this experiment contained a trace amount of N (0.27% by weight), DON removed from natural water was calculated by subtracting the measured TDN concentration from the estimated one. The estimated TDN concentration was calculated by adding the N contributed by the tannin addition to the initial TDN concentration of the natural water, the former being estimated by multiplying the N content of tannins (0.0051 mg N per mg C) by the DOC concentration increase caused by the tannin addition. The incubations were conducted in duplicate. Note that more than 90% of TDN in the water samples used was in DON form and that tannins are not known to form complexes with inorganic N.

Structural alteration of tannins (H4)

A 1.2-mL aliquot of tannin stock solution (2,000 mg L^{-1}) was mixed with 120 mL of natural waters collected

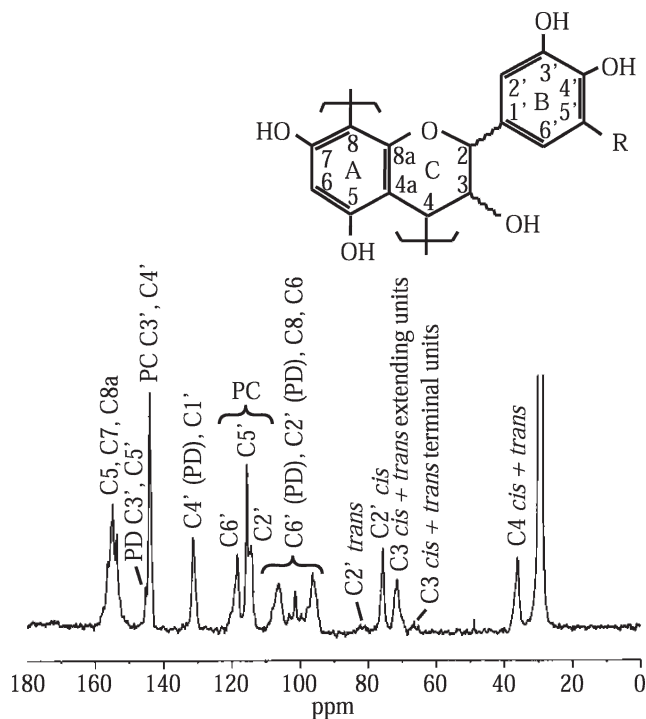


Fig. 2. Solution ^{13}C NMR spectrum of purified tannins used in a series of experiments with assignment of chemical shifts. R=H, procyanidin (PC); R=OH, prodelphinidin (PD).

within the FCE-LTER (T2, T6, and T10) in a 150-mL glass beaker (inner diameter, 50 mm) covered with a quartz plate or in an amber high density polyethylene (HDPE) Nalgene bottle and incubated under light and dark conditions, respectively, for designated periods (0, 0.5, 1, 2, 4, and 7 d). The light incubation was conducted under solar-simulated light produced by a Suntest XLS+ solar simulator (Atlas Material Testing Technology LLC) set at 765 W m^{-2} (about 1.2 times of solar noon in Miami, $25^{\circ}45'\text{N}$, $80^{\circ}22'\text{W}$). Thus, a total dosing of simulated light for a continuous 7 d was estimated to correspond to that of about 25 d of sunlight in low latitudes, assuming a daytime of 8 h. After incubation, the water samples were filtered, and the DOC and total phenol concentrations and EEM fluorescence spectra were determined. An additional experiment was conducted using Milli-Q water and Gulf Stream water. In this experiment, the incubation period under dark conditions was extended to 28 d to better understand the decomposition of tannins. The incubations were conducted in duplicate.

Dissociation of tannin–protein complexes (H5)

The experimental design was the same as for H4 except that we spiked 0.3 mL of a $2,000\text{ mg L}^{-1}$ protein stock solution (bovine serum albumin; BSA) to produce tannin–protein complexes. Gulf Stream water was used in this experiment. BSA was used in this experiment since this protein is commonly used to estimate the protein-binding ability of tannins (e.g., Kraus et al. 2003). The structural variation between BSA and mangrove-leached protein might influence the binding strength of protein–tannin

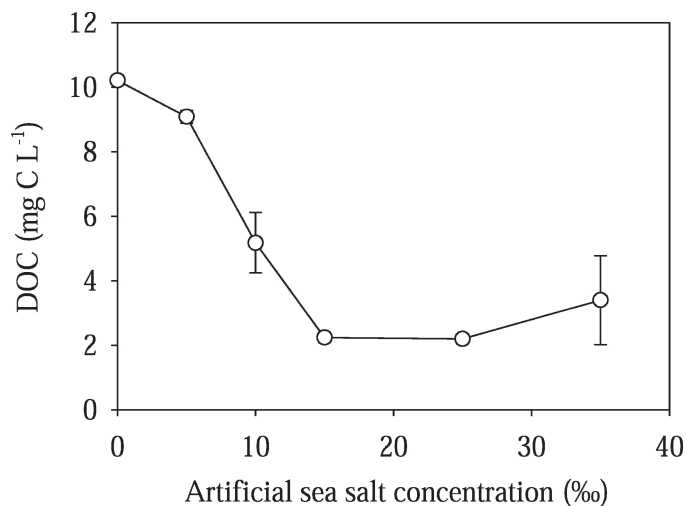


Fig. 3. Self-aggregation of tannins in artificial seawater with different salinity (H1). Tannin concentrations were measured as DOC. Symbol and error bar are the average and range of duplicates, respectively.

complexes and thereby the dissociation rate of protein from tannin–protein complexes. However, our objective in this experiment was to obtain a general idea of the influence of sunlight exposure on the stability of tannin–protein complexes. Incubation time was set for longer periods (up to 28 d) for dark incubation. DOC and TDN concentrations in the filtrate were measured. The experiment was conducted in triplicate.

Results

Structural characteristics of the purified tannins—The C and N concentrations of tannins used in a series of experiments were 53.0% and 0.27%, respectively. The solution ^{13}C NMR spectrum of the tannins (Fig. 2; with assignment of chemical shifts by Czochanska et al. [1980]) was typical of pure condensed tannins composed of flavan-3-ol units. The average number of eight monomer units was estimated from the ratio of C3 extending units (67–68 ppm) and C3 terminal units (72–73 ppm). The ratio of procyanidin (PC) to prodelphinidin (PD) in tannin polymer, which differ in the oxygenation pattern of the B ring, was elucidated to 90:10 from the relative signal intensities at 145 ppm (C3' and C4' of PC) and 146 ppm (C3' and C5' of PD) (Czochanska et al. 1980).

Aggregation of tannins (H1)—The dissolved tannin concentration decreased with the increase in salinity, and at salinities above 15‰, about 75% of mixed tannins were eliminated from the aqueous phase (Fig. 3). Therefore, we expect that a major portion of tannins can be eliminated within a day through the physical aggregation process under high-saline water conditions.

Sorption of tannins to sediments (H2)—DOC concentrations increased proportionally to the amount of tannins added for the treatment without peat (Fig. 4). However, for the treatment with peat, no appreciable increase was

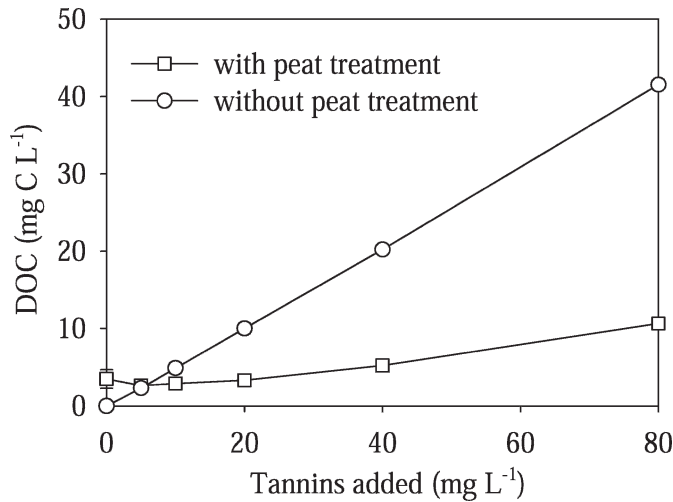


Fig. 4. Adsorption of tannins on sediment (H2): open circles are for treatments without peat (reference), and open squares are for those with peat. Tannin concentrations were measured as DOC. Symbol and error bar are the average and range of duplicates, respectively.

observed in the DOC concentration for solutions with tannin concentrations below 20 mg L⁻¹, and more than 80% of the added tannins were adsorbed at 80 mg L⁻¹. The sorption of tannins to peat was fast, occurring in less than 0.5 h after mixing (data not shown).

Coprecipitation of tannins with DON in natural water (H3)—Although the DOC concentrations increased as the amount of spiked tannins increased, the rate was lower than that estimated from the amount of tannins added, indicating the precipitation of a portion of the added tannins (Fig. 5a). A higher precipitation rate for T10, compared with T2 and T6, waters was attributed to their high salinity (*see H1*). While DOC data did not prove the occurrence of the coprecipitation of tannins with DOM in natural waters, TDN data (Fig. 5b,c) demonstrated clearly that the addition of tannins at a level of 20 mg L⁻¹ sequestered TDN in natural water by 0.05–0.08 mg N L⁻¹, or 8–17%.

Structural alteration of tannins (H4)—DOC concentrations fluctuated but did not show any consistent trend for T2 and T6 under dark incubations over a 7-d period (Fig. 6). In contrast, they decreased to 46–64% of 0-d concentrations, followed by a slight increase under light conditions. A quick decrease in the DOC concentration by ca. 40% was observed under dark and light conditions for T10 as a result of aggregation (*see H1*), which was followed by an increase under light conditions.

The ratio of absorption at 300 nm and 350 nm (A_{300}/A_{350}) decreased throughout the dark incubation, while it first decreased and then increased for the light incubation (Fig. 7). The fluorescence emission peak intensity (Ex/Em = 280/314 nm), which was used as a proxy for nonaltered tannins, decreased soon after incubation for both dark and light exposure (Fig. 8). The decrease was faster under light exposure than under dark exposure for all of the water

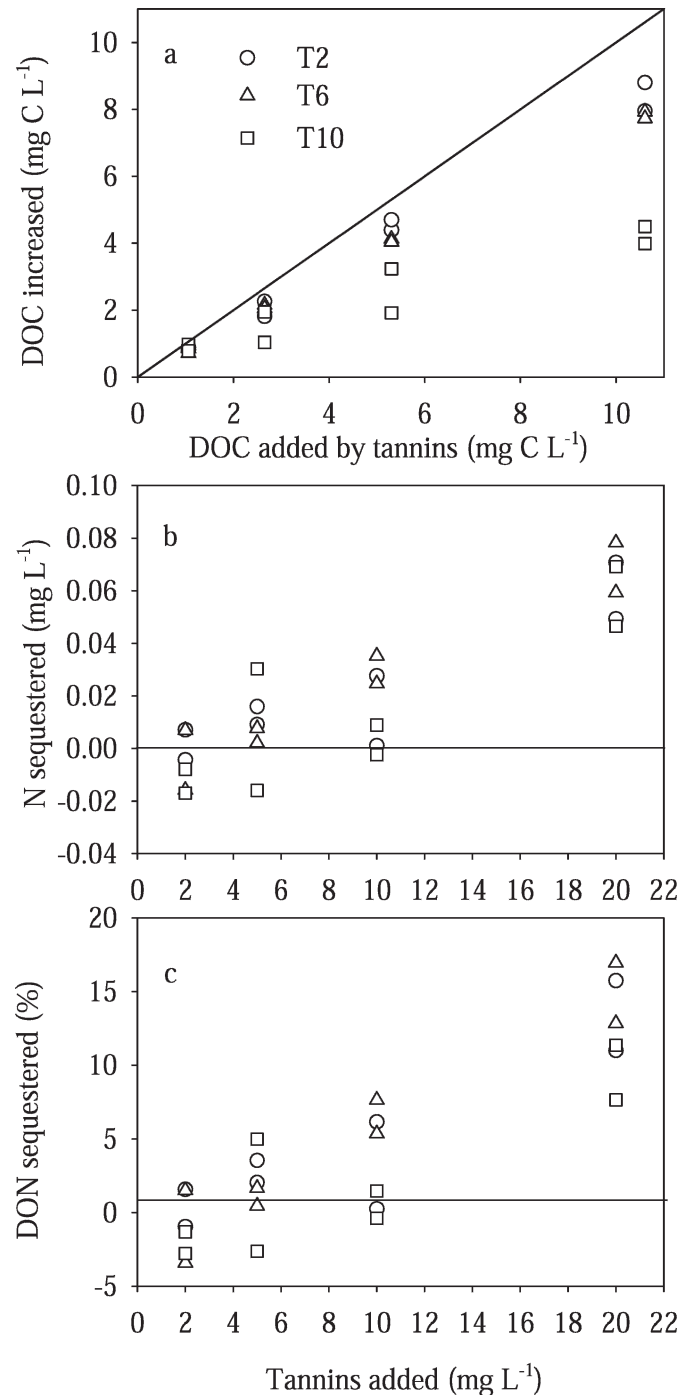


Fig. 5. Sequestration of dissolved organic matter in natural water by tannins (H3): (a) self-/co-aggregation of tannins measured by DOC; (b) DON sequestration by tannins shown in mg N L⁻¹; (c) DON sequestration by tannins shown as % of original DON. The circle, triangle, and square symbols refer to T2 (freshwater marsh), T6 (mangrove swamp), and T10 (estuarine), respectively. Symbols will be the same in subsequent figures. Lines in figures represent theoretical values with no sequestration. Since tannins contained trace amount of N, DON sequestration was calculated by subtracting the measured value from the expected value.

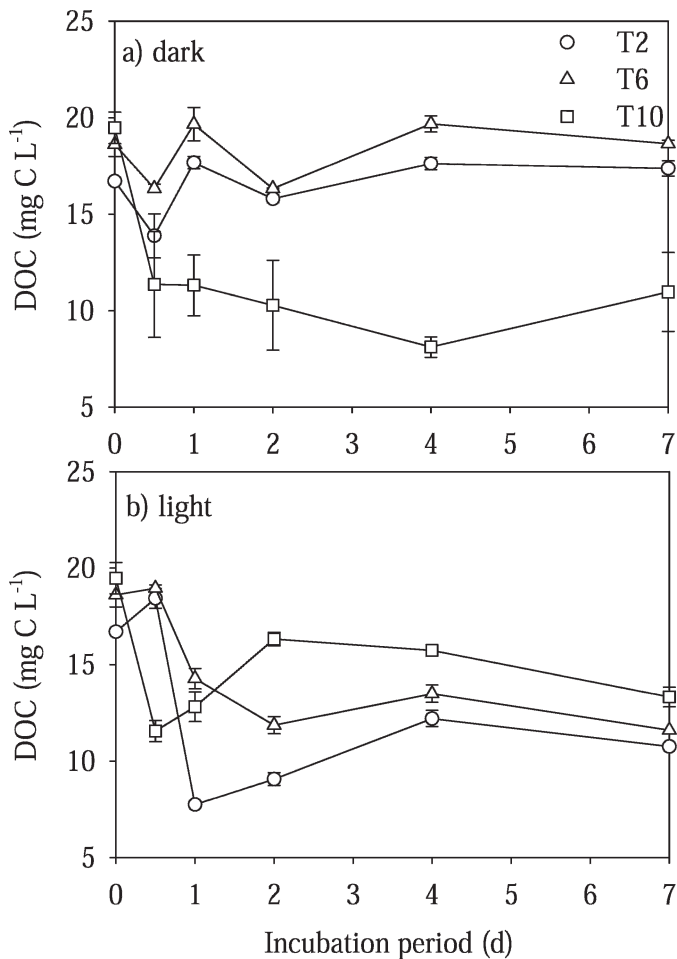


Fig. 6. Change in the DOC concentrations of natural water that was mixed with tannins and incubated under (a) dark and (b) light conditions (H4). The circle, triangle, and square symbols refer to T2 (freshwater marsh), T6 (mangrove swamp), and T10 (estuarine), respectively. The symbols and error bars represent the average of duplicate analyses and the range, respectively.

samples. These results indicate that the decrease of tannin signal is not only due to physical removal of tannins but also to the chemical alteration of the tannin structure.

When tannins were incubated in Gulf Stream water, the DOC concentration decreased soon after the start of the dark incubation and remained at a low level until the end of the incubation period (Fig. 9a). In contrast, the DOC concentration increased after 0.5 d of incubation under light conditions. Tannin concentrations detected by fluorescence decreased rapidly, with a half-life of less than 1 d for both treatments (Fig. 9b). When tannins were incubated in Milli-Q water, the DOC concentration decreased by <15% and by ca. 30% after 28 d of dark and 7 d of light incubations, respectively (Fig. 9a). The tannin concentration detected by fluorescence decreased gradually to 50% after 28 d of incubation under dark conditions, but it decreased rapidly after light exposure (half-life being less than a day; Fig. 9b). The trend in the decrease of tannin concentration measured by the Folin–Ciocalteu method

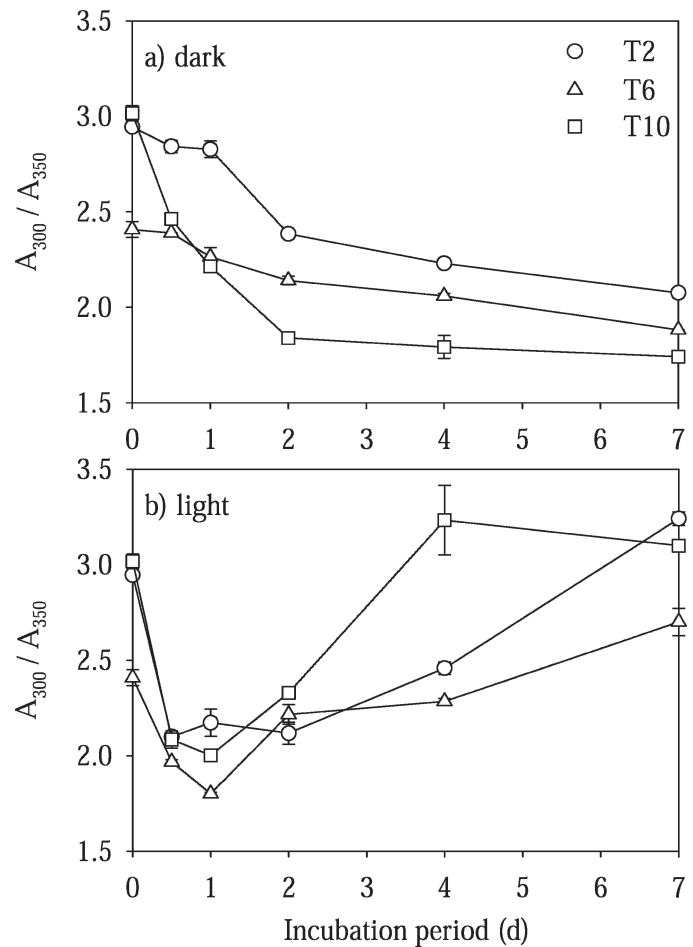


Fig. 7. Change in the A_{300}/A_{350} values of natural water that was mixed with tannins and incubated under (a) dark and (b) light conditions (H4). The circle, triangle, and square symbols refer to T2 (freshwater marsh), T6 (mangrove swamp), and T10 (estuarine), respectively. The symbols and error bars represent the average of duplicate analyses and the range, respectively.

was similar to that detected by the fluorescence method, although the latter was more rapid (Fig. 9c).

The EEM fluorescence spectra of the diagenetic products of tannins in Gulf Stream water differed for samples incubated under dark versus light conditions (Fig. 10). Tannin freshly dissolved into Milli-Q water showed a single peak at Ex/Em = 280/314 nm (Fig. 10a). However, because of their high reactivity in natural waters, partial change in the chemical structure was already apparent for tannins freshly dissolved in Gulf Stream water (EEM spectra were measured within 2 h after dissolution) (Fig. 10b). The EEM properties of tannin solutions changed further during incubation under both dark and light conditions; a dominant peak was observed at Ex/Em = 430/598 nm after 1 d, which disappeared and was replaced by peaks at <260/470 nm and 385/479 nm after 7 d (Fig. 10c,e). The position of the latter peak shifted slightly to a shorter wavelength (Ex/Em = 370/470 nm) after 28 d (Fig. 10g). The major peaks of tannin solution incubated under light were observed at Ex/Em = <260/468 nm, 305/461 nm, and 365/469 nm after 1 d of incubation (Fig. 10d). The position

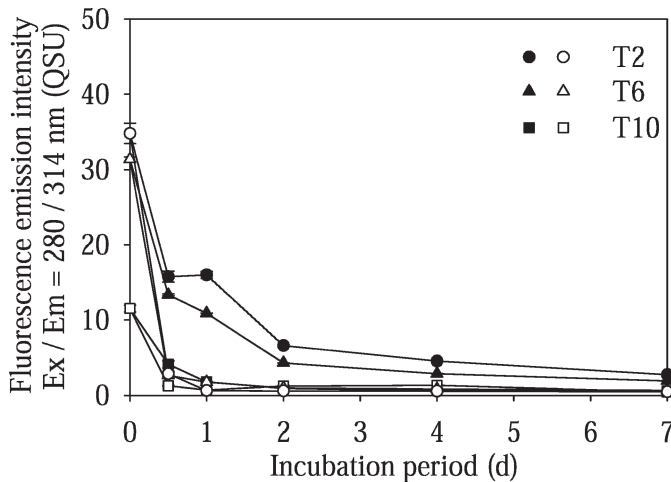


Fig. 8. Change in the tannin concentration in natural water incubated under dark and light conditions (H4). Tannin concentration was detected by a fluorescence emission intensity at Ex/Em = 280/314 nm. The fluorescence intensity of a corresponding water sample incubated without tannins for the same period was subtracted as a blank. Closed and open symbols are for treatments incubated under dark and light conditions, respectively. The symbols and error bars represent the average of duplicate analyses and the range, respectively.

of the latter two peaks shifted to a shorter wavelength during incubation, and the intensity increased and then decreased after 2 d and 7 d, respectively (Fig. 10f,h).

Dissociation of tannin–protein complexes (H5)—The dissolved THAA concentration was lower for treatments with tannin than for those without, by 45% at 0 d (Fig. 11a,b). The dissolved THAA concentration decreased for 14 d and thereafter remained at a low level under dark conditions. The gradual decrease of THAA concentration during the incubation period was ascribed to the microbial mineralization of dissolved THAA remaining in the solution. Dissolved THAA concentration did not increase during 28 d of dark incubation for treatments with tannin, indicating the refractory nature of tannin–protein complexes. In contrast, the dissolved THAA concentration started to increase consistently after 1 d of incubation under light exposure (Fig. 11). After 7 d of exposure, the THAA concentration for treatments with tannins was similar to that without tannins.

Discussion

A series of model experiments revealed that both physical and chemical processes control the fate of tannins in aquatic environments. Physical processes include aggregation and adsorption on particulate/peat/sediment (H1 and H2). Aggregation was prominent under high salinity (H1), indicating that rain events, estuarine salinity gradients, tidal conditions, and seasonal variations in freshwater loading can influence this process. Under low salinity, adsorption of tannins on sediment will be an important process (H2). This is likely to be conditioned by pH and

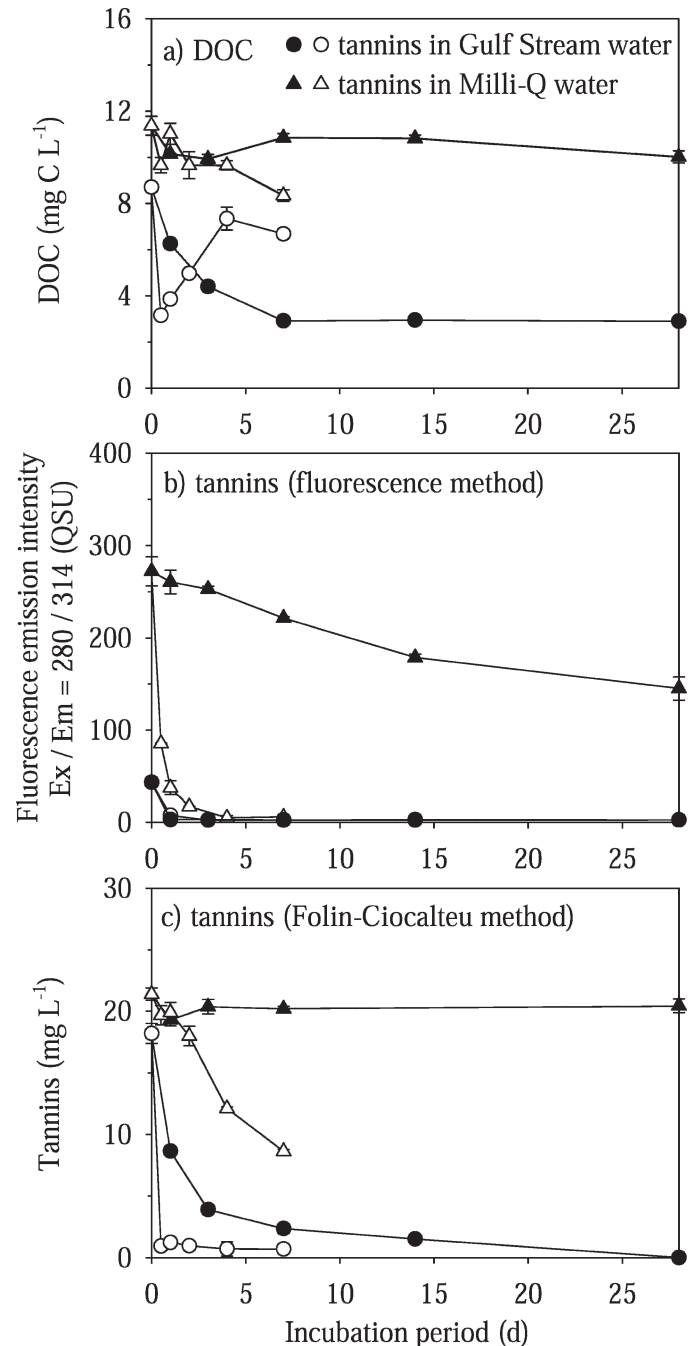


Fig. 9. Change in the (a) DOC and (b) tannin concentrations incubated in Milli-Q or Gulf Stream water under dark and light conditions (H4). Tannin concentration was detected by a fluorescence emission intensity at Ex/Em = 280/314 nm. The DOC concentration or fluorescence intensity of corresponding water samples incubated without tannins for the same period was subtracted as a blank. Circle and triangle symbols refer to tannins incubated in Milli-Q and Gulf Stream waters, respectively. Closed and open symbols refer to tannins incubated under dark and light conditions, respectively. The symbols and error bars represent the average of duplicate analyses and the range, respectively.

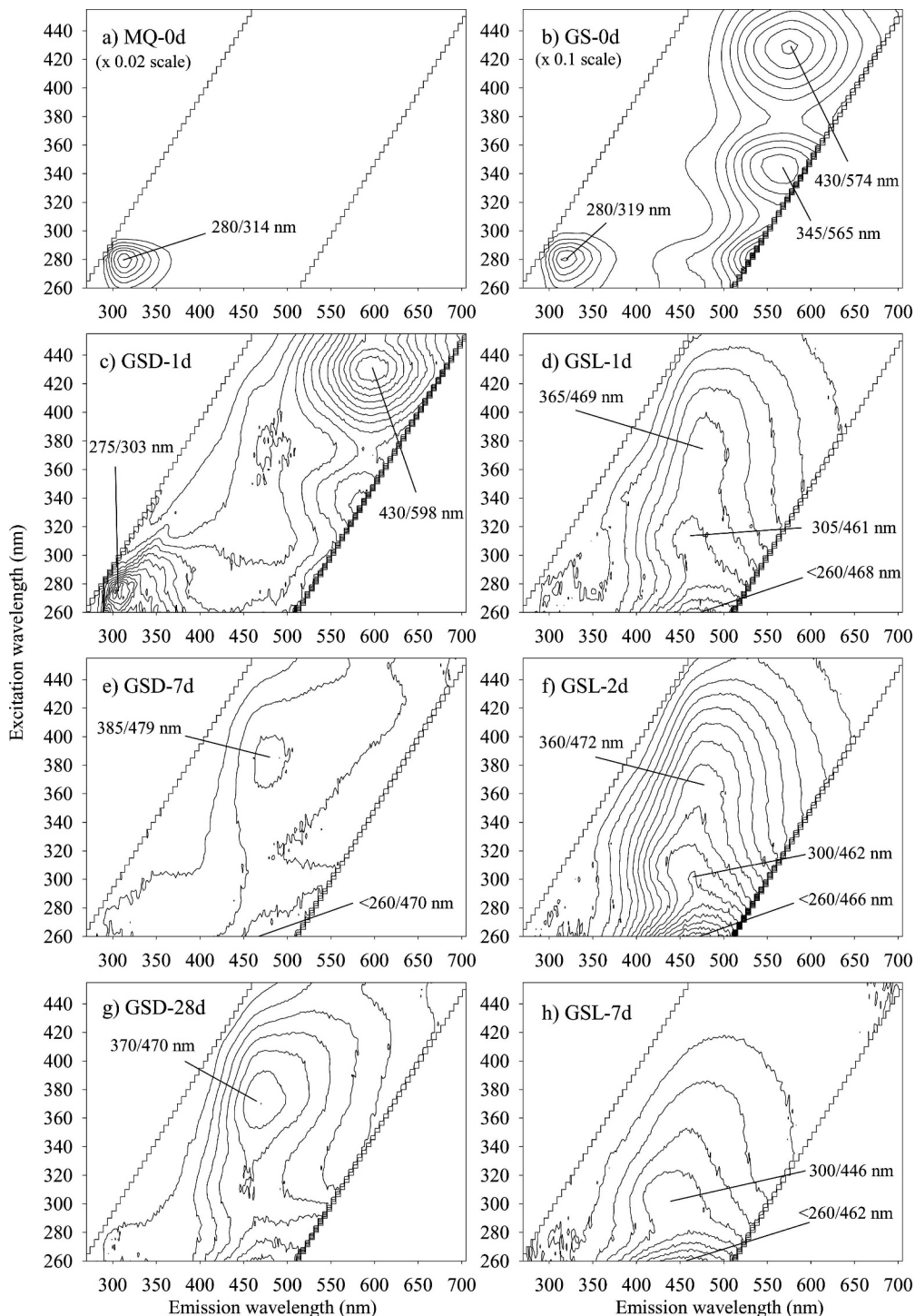


Fig. 10. Change in the EEM spectra of tannin solution incubated under dark and light conditions (H4). (a) 0 d in Milli-Q water (MQ); (b) 0 d in Gulf Stream water (GS); (c, e, g) 1 d, 7 d, and 28 d after incubation in Gulf Stream water under dark conditions (GSD), respectively; (d, f, h) 1 d, 2 d, and 7 d after incubation in Gulf Stream water under light conditions (GSL), respectively. Background EEM spectra of GS water, which was incubated for the same period without tannins, were subtracted from each EEM spectra.

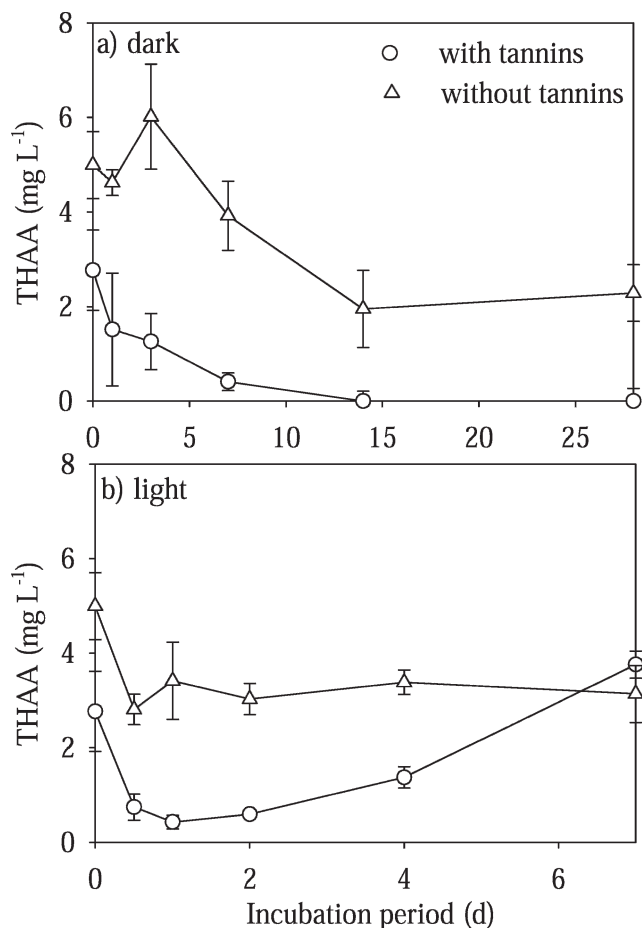


Fig. 11. Change in the THAA concentration in Gulf Stream water mixed with proteins (and tannins) and incubated under (a) dark and (b) light conditions (H5). The circle and triangle symbols refer to the treatments incubated with and without tannin, respectively. Error bars are the standard deviation of triplicate analyses.

peat/sediment characteristics, such as quality and quantity of organic matter (Kaiser 2003) and clay minerals, including sesquioxides (Varadachari et al. 1994; Kaal et al. 2005), grain size, and surface area (Kiem and Kögel-Knabner 2002).

Tannins were also found to be chemically reactive in natural waters, and they undergo a two-stage diagenesis, polymerization followed by depolymerization in aquatic environments soon after leaching (H4). Light seems to initially stimulate polymerization of tannins, but it brings about depolymerization when tannins are exposed extensively. This is indicated by the V-shaped, time-based distribution of DOC concentration and the A_{300}/A_{350} value for samples incubated in natural waters under light conditions (Figs. 6, 7, 9). It is noteworthy that precipitated tannins can become soluble again after a certain degree of decomposition and contribute to the DOC pool in coastal estuarine water. This bidirectional change was not observed for tannins incubated in Milli-Q water under light. Instead they decreased throughout the 7-d incubation (result not shown for A_{300}/A_{350}). As such, the data indicate that the

diagenetic progresses were slower in Milli-Q water and that the polymerization of tannins seems to be the dominant process. The difference in the reactivity of tannins in natural waters may thus result from differences in pH, trace-metal concentrations (especially transition metals; Shindo and Huang 1984), and coexisting DOM quality and quantity (Sandvik et al. 2000).

The contrasting nature (polymerization vs. depolymerization) in the reactivity of light-exposed DOM has been observed by several researchers and attributed to differences in the quality of DOM (reviewed by Moran and Covert 2003; Anesio et al. 2005). Our results indicate that the total dosing of light and water quality will also affect the fate of tannins. The half-life of “detectable tannins” in natural water, as determined by fluorescence (Ex/Em = 280/314 nm) and the Folin–Ciocalteu method, was less than 1 d, regardless of the exposure to light (Figs. 8, 9b,c). The results indicate that a major portion of tannins leached from mangrove leaves is being processed (transformed/eliminated) within a day in aquatic environments through biotic and/or abiotic chemical reactions. Differences in the levels of decrease of tannins detected by the two methods are due to the difference in the mechanisms involved in the detection (i.e., changes in the molecular orbitals for fluorescence vs. changes in reducing power for the Folin–Ciocalteu method).

It is notable that the EEM spectra of diagenetic products of tannins had fluorescence signals overlapping with the visible humic-like peak (peak C; Ex/Em, 320–360/420–460 nm) and marine humic-like peak (peak M; 290–310/370–410 nm) according to the classification proposed by Coble et al. (1998) (Fig. 10). The peak position of the fluorescence signal observed near the peak M was, however, shifted to a longer wavelength compared to the typical peak M position. Peaks C and M are considered to originate from terrestrial and marine sources, respectively. Our results indicate that diagenetic products of tannins can contribute to peak C, and also peak M after extensive exposure to light, and as such may be important sources of these peaks in tropical and subtropical estuaries and coastal regions.

Mangrove roots sometimes associate with mycorrhizae (Sengupta and Chaudhuri 2002; Kothamasi et al. 2006). Despite the refractive character of tannin–protein complexes against microbial degradation, some mycorrhizae can produce exoenzymes that degrade tannin–protein complexes and utilize the released N (Bending and Read 1996; Wu et al. 2003). Therefore, mangroves might be able to utilize the N in tannin–protein complexes through their symbiotic mycorrhizae.

Furthermore, insoluble tannin–protein complexes degrade slowly under exposure to sunlight, releasing previously bound proteins into the aquatic environment (Fig. 11; H5). Therefore, the tannin–protein complexes may serve as a long-lasting source of N in such ecosystems, which may improve synchrony between supply of N and its uptake by mangroves. This process will be quicker in open estuaries, where exposure to sunlight is more intense. In mangrove forests, the dense canopy attenuates sunlight intensity reaching the forest floor. The degree of light

penetration, and therefore the rate of protein release, will be influenced by gaps in the canopy caused by low biomass density, tree mortality, and natural disturbances such as hurricanes, as well as defoliation. In the hypothetical case of the absence of tannins, DON concentration in the aquatic ecosystems could fluctuate more as the result of an intermittent release of DON each time abscised leaves are submerged. This DON would not be used as efficiently by mangroves or stored in soils, but would be most likely consumed by microbes and/or exported from the mangrove ecosystem through tidal exchange.

In many oligotrophic ecosystems, N is a major limiting nutrient, and, therefore, efficient N utilization is a key survival strategy. It has been reported that specific plants growing on infertile soils produce tannins to enhance the efficient and prioritized use of N (Northup et al. 1998; Preston 1999; Verkaik et al. 2006). Our results indicate that mangrove ecosystems may benefit from the production and release of tannins, leading to a more efficient use/cycling of N (H3 and H5). This will be particularly important during the early stage of the diagenesis of mangrove leaves, when significant amounts of proteins are leached (Benner et al. 1990; Davis et al. 2003). Indeed, in the latter stage of the diagenesis of mangrove leaves, another mechanism—microbial fixation of nutrients on degrading plant residue—will contribute to maintain N in mangrove forests (Rivera-Monroy and Twilley 1996; Tremblay and Benner 2006). Our findings further imply the possibility of tannin-protein complexes serving as a temporal sink of DON in and around mangrove ecosystems (H3), and these complexes might therefore influence the longer-term DON dynamics and biogeochemical processes in adjacent coastal environments.

The concentration and molecular structure of tannins vary among different species of mangrove, and our model experiments do not cover all the possible environmental variables that may affect tannin and DON dynamics. For this reason, further studies are needed to investigate the significance of tannins in DON cycling in mangrove ecosystems. However, this is the first indication of the possible importance and potential influence of tannins on DON cycling in mangrove swamps and potentially other coastal wetlands.

References

- ANESIO, A. M., W. GRANÉLI, G. R. AIKEN, D. J. KIEBER, AND K. MOPPER. 2005. Effect of humic substance photodegradation on bacterial growth and respiration in lake water. *Appl. Environ. Microbiol.* **71**: 6267–6275.
- BALDWIN, I. T., R. K. OLSON, AND W. A. REINERS. 1983. Protein binding phenolics and the inhibition of nitrification in subalpine balsam fir soils. *Soil Biol. Biochem.* **15**: 419–423.
- BASAK, U. C., A. B. DAS, AND P. DAS. 1999. Organic constituents in leaves of 9 mangrove species of Orissa Coast, India. *Pak. J. Bot.* **31**: 55–62.
- BENDING, G. D., AND D. J. READ. 1996. Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. *Soil Biol. Biochem.* **28**: 1603–1612.
- BENNER, R., P. G. HATCHER, AND J. I. HEDGES. 1990. Early diagenesis of mangrove leaves in a tropical estuary: Bulk chemical characterization using solid-state ^{13}C NMR and elemental analyses. *Geochim. Cosmochim. Acta* **54**: 2003–2013.
- BRADLEY, R. L., B. D. TITUS, AND C. M. PRESTON. 2000. Changes to mineral N cycling and microbial communities in black spruce humus after additions of $(\text{NH}_4)_2\text{SO}_4$ and condensed tannins extracted from *Kalmia angustifolia* and *balsam fir*. *Soil Biol. Biochem.* **32**: 1227–1240.
- CASTELL, J. V., M. CERVERA, AND R. MARCO. 1979. A convenient micromethod for the assay of primary amines and proteins with fluorescamine. A reexamination of the conditions of reaction. *Anal. Biochem.* **99**: 379–391.
- COBLE, P. G., C. E. DEL CASTILLO, AND B. AVRIL. 1998. Distribution and optical properties of CDOM in the Arabian Sea during the 1995 Southwest Monsoon. *Deep-Sea Res. II* **45**: 2195–2223.
- COMLEY, B. W. T., AND K. A. MCGUINNESS. 2005. Above- and below-ground biomass, and allometry, of four common northern Australian mangroves. *Aust. J. Bot.* **53**: 431–436.
- CZOCHANSKA, Z., L. Y. FOO, R. H. NEWMAN, AND L. J. PORTER. 1980. Polymeric proanthocyanidins. Stereochemistry, structural units, and molecular weight. *J. Chem. Soc. Perkin Trans. 1*: 2278–2286.
- DAVIS, S. E., C. CORRONADO-MOLINA, D. L. CHILDERS, AND J. W. DAY. 2003. Temporally dependent C, N, and P dynamics associated with the decay of *Rhizophora mangle* L. leaf litter in oligotrophic mangrove wetlands of the Southern Everglades. *Aquat. Bot.* **75**: 199–215.
- DE LEEUW, J. W., AND C. LARGEAU. 1993. A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal, and petroleum formation, p. 23–72. *In* M. H. Engel and S. A. Macko [eds.], *Organic geochemistry: Principles and applications*. Plenum.
- DITTMAR, T., N. HERTKORN, G. KATTNER, AND R. J. LARA. 2006. Mangroves, a major source of dissolved organic carbon to the oceans. *Glob. Biogeochem. Cycles* **20**: GB1012, doi:10.1029/2005GB002570.
- FOREST, K., P. WAN, AND C. M. PRESTON. 2004. Catechin and hydroxybenzhydrols as models for the environmental photochemistry of tannins and lignins. *Photochem. Photobiol. Sci.* **3**: 463–472.
- FRANKOVICH, T. A., AND R. D. JONES. 1998. A rapid, precise and sensitive method for the determination of total nitrogen in natural waters. *Mar. Chem.* **60**: 227–234.
- GREMM, T. J., AND L. A. KAPLAN. 1998. Dissolved carbohydrate concentration, composition, and bioavailability to microbial heterotrophs in stream water. *Acta Hydrochim. Hydrobiol.* **26**: 167–171.
- HÄTTENSCHWILER, S., AND P. M. VITOUSEK. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol. Evol.* **15**: 238–243.
- HERNES, P. J., AND J. I. HEDGES. 2004. Tannin signatures of barks, needles, leaves, cones, and wood at the molecular level. *Geochim. Cosmochim. Acta* **68**: 1293–1307.
- KAAL, J., K. G. J. NIEROP, AND J. M. VERSTRATEN. 2005. Retention of tannic acid and condensed tannin by Fe-oxide-coated quartz sand. *J. Coll. Interface Sci.* **287**: 72–79.
- KAISER, K. 2003. Sorption of natural organic matter fractions to goethite ($\alpha\text{-FeOOH}$): Effect of chemical composition as revealed by liquid-state C-13 NMR and wet-chemical analysis. *Org. Geochem.* **34**: 1569–1579.

- KIEM, R., AND I. KÖGEL-KNABNER. 2002. Refractory organic carbon in particle-size fractions of arable soils II: Organic carbon in relation to mineral surface area and iron oxides in fractions <6 μm . *Org. Geochem.* **33**: 1699–1713.
- KOTHAMASI, D., S. KOTHAMASI, A. BHATTACHARYYA, R. C. KUHAD, AND C. R. BABU. 2006. Arbuscular mycorrhizae and phosphate solubilising bacteria of the rhizosphere of the mangrove ecosystem of Great Nicobar Island, India. *Biol. Fertil. Soils* **42**: 358–361.
- KRAUS, T. E. C., R. A. DAHLGREN, AND R. J. ZASOSKI. 2003. Tannins in nutrient dynamics of forest ecosystems—a review. *Plant Soil* **256**: 41–66.
- KUITERS, A. T. 1990. Role of phenolic substances from decomposing forest litter in plant-soil interactions. *Acta Bot. Neerl.* **39**: 329–348.
- KUMADA, K. 1987. *Chemistry of soil organic matter*. Elsevier.
- LIN, Y.-M., J. W. LIU, P. XIANG, P. LIN, G. F. YE, AND L. D. S. L. STERNBERG. 2006. Tannin dynamics of propagules and leaves of *Kandelia candel* and *Bruguiera gymnorrhiza* in the Jiulong River Estuary, Fujian, China. *Biogeochemistry* **78**: 343–359.
- MAIE, N., A. BEHRENS, H. KNICKER, AND I. KÖGEL-KNABNER. 2003. Changes in the structure and protein binding ability of condensed tannins during decomposition of fresh needles and leaves. *Soil Biol. Biochem.* **35**: 577–589.
- , R. JAFFÉ, T. MIYOSHI, AND D. L. CHILDERS. 2006a. Quantitative and qualitative aspects of dissolved organic carbon leached from plants in an oligotrophic wetland. *Biogeochemistry* **78**: 285–314.
- , AND OTHERS. 2006b. Chemical characteristics of dissolved organic nitrogen in an oligotrophic subtropical coastal ecosystem. *Geochim. Cosmochim. Acta* **70**: 4491–4506.
- McKNIGHT, D. M., E. W. BOYER, P. K. WESTERHOFF, P. T. DORAN, T. KULBE, AND D. T. ANDERSEN. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol. Oceanogr.* **46**: 38–48.
- MORAN, M. A., AND J. S. COVERT. 2003. Photochemically mediated linkages between dissolved organic matter and bacterioplankton, p. 243–262. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], *Aquatic ecosystems: Interactivity of dissolved organic matter*. Academic Press.
- NIEROP, K. G. J., C. M. PRESTON, AND J. M. VERSTRATEN. 2006. Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization. A case study using four tannins. *Soil Biol. Biochem.* **38**: 2794–2802.
- NORTHUP, R. R., R. A. DAHLGREN, AND J. G. MCCOLL. 1998. Polyphenols as regulators of plant-litter-soil interactions in Northern California's pygmy forest: A positive feedback? *Biogeochemistry* **42**: 189–220.
- ODUM, W. E., AND E. J. HEALD. 1975. Mangrove forests and aquatic productivity, p. 129–136. *In* A. D. Hasler [ed.], *Coupling of land and water systems*. Springer-Verlag.
- ONG, J. E. 1993. Mangroves—a carbon source and sink. *Chemosphere* **27**: 1097–1107.
- PRESTON, C. M. 1999. Condensed tannins of salal (*Gaultheria Shallon* Pursh): A contributing factor to seedling “growth check” on northern Vancouver Island?, p. 825–841. *In* G. G. Gross, R. W. Hemingway and T. Yoshida [eds.], *Plant Polyphenols 2—chemistry, biology, pharmacology, ecology*. BASIC LIFE SCIENCES, v. 66. Kluwer Academic/Plenum.
- RIVERA-MONROY, V. H., AND R. R. TWILLEY. 1996. The relative role of denitrification and immobilization in the fate of inorganic nitrogen in mangrove sediments (Términos Lagoon, Mexico). *Limnol. Oceanogr.* **41**: 284–296.
- ROBERTSON, A. I., D. M. ALONGI, AND K. B. BOTO. 1992. Food chains and carbon fluxes, p. 293–326. *In* A. I. Robertson and D. M. Alongi [eds.], *Tropical mangrove ecosystems*. Coastal and Estuarine Sciences. American Geophysical Union.
- SANDVIK, S. L. H., P. BILSKI, J. D. PAKULSKI, C. F. CHIGNELL, AND R. B. COFFIN. 2000. Photogeneration of singlet oxygen and free radicals in dissolved organic matter isolated from the Mississippi and Atchafalaya River plumes. *Mar. Chem.* **69**: 139–152.
- SCHIMEL, J. P., R. G. CATES, AND R. RUESS. 1998. The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. *Biogeochemistry* **42**: 221–234.
- SCHOFIELD, J. A., A. E. HAGERMAN, AND A. HAROLD. 1998. Loss of tannins and other phenolics from willow leaf litter. *J. Chem. Ecol.* **24**: 1409–1421.
- SCULLY, N. M., N. MAIE, S. K. DAILEY, J. N. BOYER, R. D. JONES, AND R. JAFFÉ. 2004. Early diagenesis of plant-derived dissolved organic matter along a wetland, mangrove, estuary ecotone. *Limnol. Oceanogr.* **49**: 1667–1678.
- SENGUPTA, A., AND S. CHAUDHURI. 2002. Arbuscular mycorrhizal relations of mangrove plant community at the Ganges River estuary in India. *Mycorrhiza* **12**: 169–174.
- SHINDO, H., AND P. M. HUANG. 1984. Catalytic effects of Mn(IV), Fe(III), Al and Si oxides on the formation of phenolic polymers. *Soil Sci. Soc. Am. J.* **48**: 927–934.
- TREMBLAY, L., AND R. BENNER. 2006. Microbial contributions to N-immobilization and organic matter preservation in decaying plant detritus. *Geochim. Cosmochim. Acta* **70**: 133–146.
- TWILLEY, R. R., A. E. LUGO, AND C. PATTERSON-ZUCCA. 1986. Litter production and turnover in basin mangrove forests in southwest Florida. *Ecology* **67**: 670–683.
- VALIELA, I., J. L. BOWEN, AND J. K. YORK. 2001. Mangrove forests: One of the world's threatened major tropical environments. *BioScience* **51**: 807–815.
- VARADACHARI, C., A. H. MONDAL, D. C. NAYAK, AND K. GHOSH. 1994. Clay-humus complexation—effect of pH and the nature of bonding. *Soil Biol. Biochem.* **26**: 1145–1149.
- VERKAIK, E., A. G. JONGKIND, AND F. BERENDSE. 2006. Short-term and long-term effects of tannins on nitrogen mineralisation and litter decomposition in kauri (*Agathis australis* (D. Don) Lindl.) forests. *Plant Soil* **287**: 337–345.
- WATERMAN, P. G., AND S. MOLE. 1994. *Analysis of phenolic plant metabolites*. Blackwell Scientific Publications.
- WEISS, M., AND M. SIMON. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: The relative significance of amino acids and carbohydrates. *Aquat. Microb. Ecol.* **17**: 1–12.
- WU, T. H., J. N. SHARDA, AND R. T. KOIDE. 2003. Exploring interactions between saprotrophic microbes and ectomycorrhizal fungi using a protein-tannin complex as an N source by red pine (*Pinus resinosa*). *New Phytol.* **159**: 131–139.

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