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An exploration of how litter controls drainage water DIN, DON and DOC dynamics in freely draining acid grassland soils

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Abstract Surface and subsurface litter fulfil many functions in the biogeochemical cycling of C and N in terrestrial ecosystems. These were explored using a microcosm study by monitoring dissolved inorganic nitrogen (DIN) (NH_4^+–N + NO_3^-–N), dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) concentrations and fluxes in drainage water under ambient outdoor temperatures. Subsurface litter remarkably reduced the DIN concentrations in winter, probably by microbial N uptake associated with higher C:N ratio of added litter compared with soil at 10–25 cm depth. Fluxes of DIN were generally dominated by NO_3^-–N; but NH_4^+–N strongly dominated DIN fluxes during freeze–thaw events. Appreciable concentrations of NH_4^+–N were observed in the drainage from the acid grassland soils throughout the experiment, indicating NH_4^+–N mobility and export in drainage water especially during freeze–thaw. Litter contributed substantially to DOC and DON production and they were correlated positively ($p < 0.01$) for all treatments. DOC and DON concentrations correlated with temperature for the control ($p < 0.01$) and surface litter ($p < 0.001$) treatments and they were higher in late summer. The subsurface litter treatment, however, moderated the effect of temperature on DOC and DON dynamics. Cumulative N species fluxes confirmed the dominance of litter as the source of DON and DOC in the drainage water. DON constituted 42, 46 and 62% of cumulative TDN flux for control, surface litter and subsurface litter treatments respectively.

Keywords Litter · Dissolved organic carbon (DOC) · Dissolved organic nitrogen (DON) · Dissolved inorganic nitrogen (DIN) · Seasonal variations · Grassland

Introduction

Litter layers provide an integral link in the interactive biogeochemical cycles of C and N in natural and semi-natural ecosystems. They exert multidirectional effects on soil physico-chemical properties, on nutrient element cycling and bioavailability, and on soil faunal diversity and composition (Hättenschwiler et al. 2005; Sayer 2006). Litter biodegradation is generally considered a key factor to the availability of N, P, S and other nutrients, and hence regulates fertility and primary productivity within a natural ecosystem (Kuperman 1999; Henry et al. 2008). Soil organic matter (SOM) forms a fundamental nutrient pool in grasslands, where litter deposition and decomposition provide an energy flow continuum and essential nutrients from and for biogeochemical transformations (Dubeux et al. 2007).

Litter decomposition releases dissolved organic nitrogen (DON) and dissolved organic carbon (DOC)

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into mineral soils (Homann and Grigal 1992; Kalbitz et al. 2000). Subsequent mineralisation of the organic N into bioavailable inorganic N (NH_4^+–N and NO_3^-–N) is regarded a key process for soil-based N release (Davidson et al. 1992).

There is extensive evidence that fresh, recently added litter and plant residues are major sources of DOC which percolates into mineral soil layers (Qualls et al. 1991; Kaiser et al. 1997; Kalbitz et al. 2000). Once in mineral soil layers, DOC can be exported to ground and surface waters, act as a metabolite for microbes, or be retained on soil mineral surfaces by abiotic mechanisms, predominantly sorption to soil particles (Kalbitz et al. 2000; Nieder and Benbi 2008). These factors were considered to explain the decline in DOC concentrations with depth (Qualls and Haines 1992; McCracken et al. 2002). DOC has well-established ecological significance as it may play a key role in translocation, especially for the nutrient elements N, P and S (Kaiser et al. 2001), metals (Tipping 2002) and organic pollutants (Chiou et al. 1986). Lavelle et al. (1993) prioritized three factors controlling litter decomposition: climate > litter > soil organisms. Among the climatic factors, seasonal variations in temperature and moisture can have pronounced effects on DOC concentrations and fluxes. There is strong evidence that increase in temperature enhances the concentrations and fluxes of DOC leached (Gödde et al. 1996; Andersson et al. 2000). Field studies in regions with seasonal temperature and precipitation patterns similar to those of the area for the present study have therefore demonstrated the highest DOC concentrations in summer and autumn (Michalzik and Matzner 1999; Solinger et al. 2001).

In many studies the concentration of dissolved inorganic nitrogen (DIN) (NO_3^-–N + NH_4^+–N) leached is higher than that of DON (Khanna 1981), largely due to many measurements having been made in heavily N-fertilized catchments. Predominately NO_3^-–N is the major form of DIN exported out of soil profiles, especially from heavily N-impacted ecosystems which have attained an N-saturated state (Aber et al. 1998). In contrast, NH_4^+–N mobility is thought to be an insignificant contributor to DIN losses (Fernando et al. 2005). Recent investigations, however, have shown that NH_4^+–N can be substantially mobile in some ecosystems (Mian et al. 2009; Lorz et al. 2010).

With growing interest in DON measurements, it has been shown that DON may constitute up to 94% of total dissolved nitrogen (TDN) lost from deciduous forests (Qualls et al. 1991). Hawkins et al. (1997) reported that 20% of TDN was lost as DON in lysimeters draining grassland in Devon, UK. A recent study of Welsh grassland also showed that DON can lead to surface water N enrichment (Jones et al. 2004). Campbell et al. (2000) listed studies showing $\geq$50% DON contribution toward TDN fluxes in throughfall, soil solutions and stream waters. The DON mobility is largely controlled by its sorption to soil minerals, and to lesser extent by microbial uptake and biodegradation processes (Qualls and Haines 1992). However, Gregorich et al. (2003) found DON more biodegradable than DOC and reported 50% DON mineralization in 10 days. As for DOC seasonal variation, Currie et al. (1996) found increased DON concentrations during late summer in forest floor leachates in the northeastern United States and linked this to accelerated litter decomposition in summer.

There are, however, contrasting points of view in the literature about the similarity in seasonal patterns of DOC and DON dynamics. Michalzik et al. (2001) studied 42 soils in forested ecosystems and found strong positive correlation between DOC and DON fluxes. Similarly DOC and DON concentrations were highly positively correlated in stream water (Goodale et al. 2005). However, decoupling between DOC and DON has also been found, suggesting different factors at least partially regulate the dynamics of each determinant (Solinger et al. 2001). This can particularly happen, as suggested by McDowell (2003), in long-term N-manipulation studies, when N fertilization doubled the DON whereas DOC concentrations were not affected. He emphasised the need to gain insights into high variations in DOC:DON ratios in soil solution to better understand the ecological significance of DON.

Despite the widespread potential for DOC and DON to be exported to aquatic systems, most recent research has targeted only forests (e.g. Solinger et al. 2001; Park and Matzner 2006) rather than managed agricultural systems (e.g. Murphy et al. 2000) or grassland ecosystems (e.g. Ghani et al. 2007; Sanderman et al. 2008). When Kalbitz et al. (2000) reviewed extensive literature to explore the factors regulating DOC and DON dynamics, they largely discussed studies from forest ecosystems.

Very recently, Ghani et al. (2007) looked at DON in various ecosystems and its mobility into water bodies, and advocated better quantification of DON in agricultural and grassland systems. Studies of DOC dynamics in grasslands are especially rare, even although European ecosystems comprise 30% of grasslands and their contribution to C storage is similar to that of forest soils (Arrouays et al. 2001). In the conclusions to a previous study by the authors (Riaz et al. 2009) on N transformations in N-impacted grassland soils near York, UK, it was suggested that the activity of soil fauna might be influencing the systems via litter incorporation at some sites. There seemed to be significant differences between N mineralization rates if litter was incorporated naturally into subsoils rather than remaining on the soil surface.

From the preceding discussion, apparently our understanding of controls on the dynamics of DOC and DON, their mobility through mineral soils into water bodies, and their seasonal variations in the grasslands remained largely unexplored. Therefore, a litter manipulation microcosm study was conducted at outdoor ambient temperatures for a freely draining acid grassland soil near York, UK, to answer following key questions:

(1) To what extent does the presence of the litter at the surface and/or in subsurface soil of an acid grassland affect concentrations and fluxes of DIN (NH_4^+–N + NO_3^-–N), DON, TDN and DOC in drainage water?
(2) How do the seasonal changes in temperature from winter to summer affect DIN, DON and DOC dynamics associated with the presence of such litter?
(3) If considerable amounts of DIN and DON do occur in drainage water, do mobile anion (SO_4^{-2} and Cl^-) concentrations and DOC concentrations influence the mobility of DIN and DON through the soil profile into drainage water?

Materials and methods

Characteristics of the study site

Soils in the vicinity of the experimental site at Hob Moor have been described as "N-impacted" because the N pollutant deposition there exceeds 25 kg N/ha/year (Riaz et al. 2009). Soils were sampled from a freely draining, acid grassland at this Local Nature Reserve near York, UK (53°57″30′N and 1°44″8′W). Hob Moor is dominated by low fertility soils consisting of slowly permeable clay loams and more freely draining (and more acidic) very fine sandy loams to loamy sands. Perennial grasses cover the major parts of the moor, but there are some small patches of deciduous woodland. The pasture on part of the moor is grazed for ca. 6 months every summer by importing cattle for growth to keep the soils at low nutrient status and maintain high biodiversity of flora and fauna in the area. However, the soil sampling area selected for the current study is not grazed and is coarse textured and freely draining. The soils have distinct litter layers, but exhibit some observable soil faunal activities responsible for at least partial incorporation of litter into the mineral subsoil horizons.

The site has been affected by N and S deposition in the recent past (NEGTAP 2001), and is believed to receive ca. 25 kg N/ha from atmospheric deposition (Riaz et al. 2009). There is no evidence of artificial fertilizer application to the site over the last 50 years (Cresser, personal communication).

Litter and soil sampling and preparation

A 10 m × 10 m plot was selected which was visibly uniform and typical of the freely draining acid grassland, and marked out using wooden pegs in November, 2008. For the sampling of litter layer and mineral soil, ten soil profiles were excavated at random within the plot to 50 cm depth. The surface vegetation was removed gently from the protected profile face using a sharp stainless steel knife taking care to avoid disturbance to litter and underlying mineral layers.

Litter was sampled at each of the ten profiles by cutting horizontally at the exposed profile face. Intact coarse roots, stones and residual live vegetation were removed from the ten samples which were then air dried. The ten dried litter samples were passed through a 2.0-mm sieve and then thoroughly mixed to form a composite sample. Four sub-samples were taken for initial litter physico-chemical analysis. The homogenization of litter and of each mineral soil from each depth increment was deemed sufficient for triplicate microcosms to suffice for each treatment.

The mineral soils were sampled to 25 cm depth at five 5-cm increments. Soils from each depth increment from all ten pits were mixed thoroughly to form five composite incremental samples. Coarse roots and stones were removed by passing through a 2.0 mm sieve. Four sub-samples were taken for quantification of soil initial physico-chemical characteristics.

Microcosm construction and experimental set up

Experimental microcosms were constituted using the soils from the 5-sampled depth increments added in their natural sequence. The experiment included the following three treatments,

(1) Control (no litter).
(2) Surface litter (20 g litter applied at the surface, corresponding to a ca. 2 cm thick litter layer in the field).
(3) Subsurface litter (20 g litter split into three equal portions and a portion mixed with each of 0–5, 5–10 and 10–15 cm depth increment soils).

The microcosms were formed in 29 cm-long PVC pipes with a 6.4 cm inner diameter. To facilitate subsequent soil layer fractionation for destructive soil and litter analysis after specified time periods, the PVC cores were pre-lined with thin, but strong, polyvinyl acetate cylinders which were distilled-water rinsed and dried. Soils were packed to achieve consistency of densities between replicate cores by using a length of the same PVC pipe cut precisely into 5 cm lengths and capped at the bottom. Five of these 5 cm-deep cores were filled in triplicate with a soil from each sampling depth with periodic agitation to ensure uniform distribution and consistent packing for each depth increment. The mass of soil for each depth was measured in triplicate and the mean mass amount for each soil layer was used subsequently for microcosm reconstruction. Incorporation of the litter into subsoils had very little effect on the total soil volume.

Microcosms were sealed at the base with a perforated plastic cap containing 140 μm nylon mesh under a thin layer of acid-washed quartz sand to avoid direct contact between soil and the nylon mesh and loss of fine soil particles with the drainage water. The plastic caps were made airtight externally using clear adhesive tape. The microcosms were placed in large, distilled-water filled, polyethylene bags over night for wetting, then left to drain to a state assumed to be near field capacity.

The experiment comprised of three treatments, each replicated three times, with four destructive sampling dates, making 36 microcosms in total. A set of three replicates from each treatment was removed and analysed destructively after 5, 11, 21 and 31 weeks to study core N dynamics.

The experiment was conducted under outdoor ambient cold weather conditions in York from 01/12/2008 (early winter, week 1) to 06/07/2009 (mid summer, week 31). The microcosms were held vertically in large plastic boxes fitted with a supporting framework over plastic funnels draining into plastic leachate-collecting bottles covered with thick black plastic sheeting to protect them from sunlight. The boxes were kept under an open-sided roof structure to protect them from direct sunlight and rain. Air temperature was noted daily at 12:00 noon.

Simulated rain preparation, application and drainage water collection

Rain water was collected weekly for over 4 months of the previous winter period in 2007 in a pair of rain gauges at the Hob Moor, and analysed within 1–2 days of collection for calculation of an appropriate mean formulation for simulated rain. The simulated rainfall contained 0.7 mg/l of NH_4^+–N and 0.32 of mg/l NO_3^-–N which were equivalent to their weekly fluxes of 19.58 and 8.95 mg N m^{-2} respectively. The simulated rain contained no DON or DOC. A dose of 45 ml of simulated precipitation, corresponding to ca. 14 mm of rainfall, was applied to each core twice each week. Rainwater was sprinkled gently to avoid any disturbance to litter and soil layers and cores were allowed to drain freely. After the application of the second dose of simulated rain each week, a time gap of 24 h was given for cores to drain before the drainage water samples collected in pre-labelled plastic bottles were taken for analysis. Three replicates of drainage water from each treatment were analysed each week.

Analytical protocols

Litter and soil initial physico-chemical characteristics

The pH values of soil and litter were measured on field moist samples at a 1:5 m:v (soil:solution) ratio both in water and 0.5 M KCl with glass/calomel combination electrode and a pre-calibrated Thermo Orion 420 pH meter (Riaz et al. 2009). The moisture contents were determined gravimetrically by oven drying the fresh, field-moist soils to constant mass at 105°C. The oven-dried soil and litter sample residues from moisture content determinations were ground to fine powders using a Retsch MM200 ball-mill at 25 Hz for 3 min. The finely ground samples were used to determine C, N and C/N ratio on an Elementar Vario Macro CN analyser, pre-calibrated with glutamic acid. Fresh field-moist litter and soil samples were extracted with 0.5 M KCl at 1:5 m:v (soil:solution) ratio by shaking them on oscillating shaker for 2 h. The suspensions were allowed to settle for 10 min before filtering through Whatman No. 42 filter papers into pre-labelled plastic bottles that had been acid-washed, distilled water rinsed and dried. The extracts were stored at 4°C before they were analysed for NH_4^+–N and NO_3^-–N within 4 days using a Bran and Luebbe Autoanalyser-3 by a standard protocol with matrix-matched standards and reagent blanks. Soil texture was assessed by the method of Batey (1988).

Weekly drainage water analysis and flux calculations

Three drainage water samples per treatment were analysed weekly after recording their volumes. Prior to their filtration, drainage water pH was measured using a Thermo Orion 420 pH meter and glass/calomel electrode, and electrical conductivity (EC) (μS/cm) using Hanna HI 9033 Portable Conductivity Meter. The drainage water samples then were filtered through 0.45 μm Millipore membrane filters and analysed for NH_4^+–N and NO_3^-–N using a Bran and Luebbe Autoanalyser-3 and TDN on a Bran and Luebbe Autoanalyser-2 by a standard protocol with matrix-matched standards and reagent blanks. DON was calculated using the difference method [DON = TDN − (NH_4^+–N + NO_3^-–N)].

DOC concentrations in the drainage water samples were determined using an Elementar Liquitoc TOC analyser, and major anions, i.e. chloride (Cl^-), sulphate (SO_4^{-2}), fluoride (F^-) and phosphate (PO_4^{-3}), using ion chromatography (Dionex DX-120). However, F^- and PO_4^{-3} were rarely detectable, so are not included in the results and discussion sections of the manuscript. The detection limits, using the AS-14 column in the Dionex DX-120 for F^- and PO_4^{-3} were 0.05 and 0.13 μg ml respectively. The fluxes of N determinants and DOC were calculated using the output drainage water volume, and were expressed as mg N/m^2 and mg C/m^2 respectively. The cumulative input fluxes, output fluxes and their net balances in the drainage water were also calculated by summing up the individual flux values for each replicate from each treatment over 31 weeks.

Statistical analysis

DIN, DON and DOC concentrations and fluxes represent volume-weighted means ($n = 3$) for each treatment. Data were assessed for normality and, if needed, the data were log transformed for homogenization prior to analysis; however, arithmetic means of untransformed data are presented unless otherwise noted. Analysis of variance (ANOVA) was used to test for significant differences between treatments for NO_3^-–N, NH_4^+–N and DIN concentrations at specified weeks. Data were log transformed and checked for homogeneity of variance before use for ANOVA. Tukey's HSD post hoc test ($\alpha = 0.05$) was used for multiple means comparisons for significant treatment effects only. Pearson's correlation co-efficients (r) were used to study the relationships between drainage water chemical determinants. Regression analyses were performed to establish temperature effects on the DOC:DON ratio and the DON:DIN ratios. All statistical analysis was performed using SPSS 17 for Windows software.

Results

Initial physico-chemical characteristics of litter and soils

Table 1 summarises the physical and chemical properties of the litter and soils used. The soil pH (KCl) increased consistently with sampling depth

Table 1 Characteristics of the litter and soils used for the study

Soil physico-chemical properties	Litter layer	Soil mineral layers				
		0–5 cm	5–10 cm	10–15 cm	15–20 cm	20–25 cm
pH (water)	4.75 ± 0.05	4.32 ± 0.01	4.33 ± 0.00	4.35 ± 0.00	4.37 ± 0.00	4.40 ± 0.00
pH (KCl)	3.84 ± 0.05	3.29 ± 0.00	3.36 ± 0.00	3.48 ± 0.00	3.57 ± 0.00	3.61 ± 0.00
Moisture content (%)	114.4 ± 6.17	33.05 ± 1.58	23.45 ± 0.33	22.40 ± 016	21.79 ± 0.09	20.36 ± 0.00
C (%)	22.29 ± 0.93	7.97 ± 0.47	3.82 ± 0.07	3.07 ± 0.04	2.47 ± 0.05	2.61 ± 0.07
N (%)	1.26 ± 0.05	0.50 ± 0.03	0.27 ± 0.00	0.23 ± 0.00	0.19 ± 0.00	0.19 ± 0.00
C/N ratio	17.71 ± 0.09	16.08 ± 0.16	14.17 ± 0.06	13.46 ± 0.15	12.79 ± 0.08	13.44 ± 0.24
Soil texture[a]	na	LFS	LFS	LFS	FSL	FSL
KCl-extractable NH_4^+–N (mg N/kg soil)	173 ± 7.01	21.4 ± 0.47	6.30 ± 0.11	4.10 ± 0.32	3.36 ± 0.35	3.19 ± 0.37
KCl-extractable NO_3^-–N (mg N/kg soil)	2.97 ± 1.65	2.90 ± 0.34	1.95 ± 0.12	2.50 ± 1.0	1.59 ± 0.35	1.17 ± 0.15

Values are followed by ±standard error of means ($n = 4$)

na results not applicable

[a] Soil texture was assessed with hand method (*LFS* loamy fine sand, *FSL* fine sandy loam)

from 3.29 at 0–5 cm to 3.61 at 20–25 cm depth. The more organic-rich soil at 0–5 cm depth had higher moisture content than soils at 5–25 cm depth increments. In contrast to mineral soil layers, as expected, the litter layer had a 3.5 fold higher moisture content and almost threefold higher C content (Table 1). There were appreciable amounts of organic C at depth in mineral soils. Below the litter layer, which had 173 mg extractable NH_4^+–N per kg soil, extractable NH_4^+–N concentration dropped sharply and consistently from 21.4 mg/kg soil at 0–5 cm to 3.19 at 20–25 cm depth. However, KCl-extractable NO_3^-–N concentrations showed no consistent trends with depth to 25 cm. The soils were naturally freely draining with texture ranging from loamy fine sands to fine sandy loams.

Litter effects on drainage water N dynamics

Changes in NO_3^-–N concentrations

Concentrations of DIN were dominated by NO_3^-–N in the majority of the 31 weeks for all three treatments (Fig. 1). From week 2, in early winter, NO_3^-–N remained much higher in the control and surface litter treatments (Fig. 1a, b) compared with the subsurface litter treatment (Fig. 1c). However, the differences among the treatments were significant only during weeks 3, 7, 8, 10 and 14 ($p < 0.05$). During the early winter period up to week 9, belowground presence of litter (subsurface litter treatment) remarkably reduced NO_3^-–N in the drainage water (Fig. 1c). For the control and surface litter treatment (Fig. 1a, b), concentrations of NO_3^-–N declined sharply in the drainage water from the start of marked freeze–thaw events during weeks 10–12 and remained typically below 1 mg/l until week 31 in mid summer. From weeks 16 to 22, NO_3^-–N for the surface litter treatment remained below the detection limit (Fig. 1b). During weeks 15, 16 and 19 for the control treatment (Fig. 1a) and 13 and 23 for the subsurface litter treatment (Fig. 1c), NO_3^-–N remained undetectable. However, NO_3^-–N showed increasing trends from week 24 to week 30 (early to mid summer) for each treatment (Fig. 1). Concentrations of NO_3^-–N in the drainage water from the subsurface litter treatment were slightly higher between weeks 15 and 31 than those for other treatments, but the differences were never statistically significant.

Changes in NH_4^+–N concentrations

The subsurface litter treatment resulted in higher NH_4^+–N from weeks 3 to 7 (Fig. 1c) compared with the control and subsurface litter treatments (Fig. 1a, b); however, the effect was significant only during week 4 ($F = 5.202$, $p < 0.05$). Freeze–thaw cycles in weeks 10–12 had clear observable effects on NH_4^+–N for each treatment, but the surface litter treatment exhibited a much higher NH_4^+–N flush during weeks 10, 11 and 12 (Fig. 1b). The NH_4^+–N in drainage

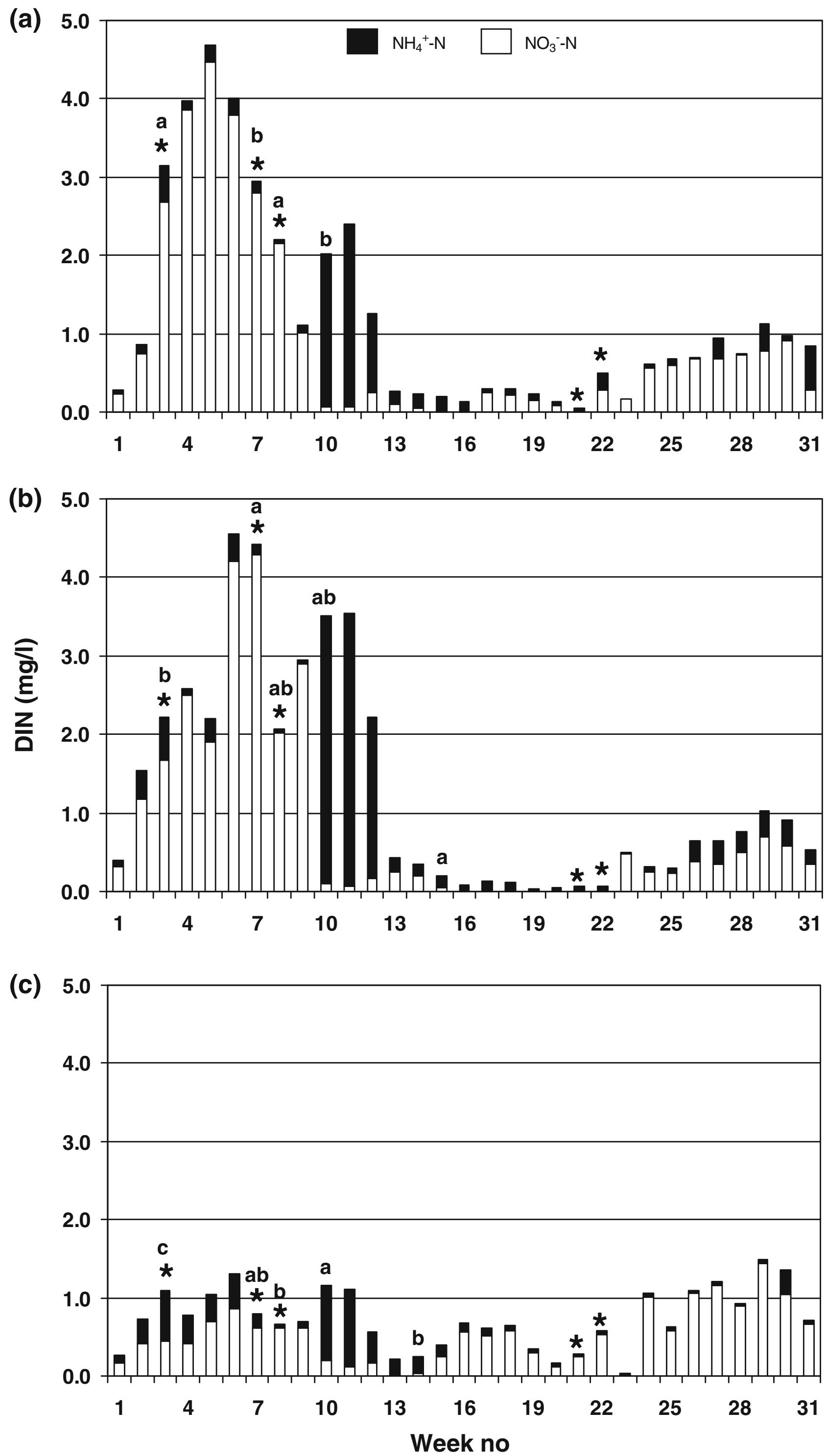

Fig. 1 DIN concentrations (mg/l) for **a** control, **b** surface litter and **c** subsurface litter treatments from week 1 (1–8 December 2008, early winter) to week 31 (30 June to 6 July 2009, mid summer). All values are means of three replicates. *Bars* with *asterisks* show significant differences for DIN between the treatments at specified weeks. *Bars* sharing *different letters* differ significantly from each other at $p < 0.05$ for NO_3^-–N concentrations at specified treatments

water only exceeded the 0.7 mg NH_4^+–N/l in rainfall during the freeze–thaw period. At all other times, NH_4^+–N concentrations were lower in drainage water than in the simulated precipitation. After the freeze–thaw event, NH_4^+–N remained below 0.5 mg/l from weeks 13 (late winter) to 31 (mid summer).

Generally, concentrations of NH_4^+–N for the surface litter treatment were higher during weeks 26, 27, 28 and 29 compared with the subsurface litter treatment. However, at week 31, NH_4^+–N (0.56 mg/l) in the control treatment was significantly higher compared with the surface and subsurface litter treatments ($F = 13.415$, $p < 0.01$). NH_4^+–N was the only form of DIN for the control during weeks 15, 16 and 21, for surface litter during weeks 16–22, and for subsurface litter during weeks 13 and 23 (Fig. 1).

Changes in DIN concentrations

Concentrations of DIN for the subsurface litter treatment (Fig. 1c) remained consistently lower than those for other treatments until week 11 (winter) and the differences were significant ($p < 0.05$) during weeks 3, 7 and 8 compared with the control and surface litter treatments (Fig. 1a, b). During the freeze–thaw events (weeks 10–12), a relatively large flush of NH_4^+–N resulted in much higher DIN production for the control and surface litter treatments compared with the subsurface litter treatment. The NH_4^+–N contributions to the DIN concentrations were relatively higher from weeks 26 to 30 (summer) for the surface litter treatment. However, for the subsurface litter treatment compared to the other treatments, from weeks 17 to 22 (spring), 24, and 26 to 30 (early to mid summer), DIN remained higher in the drainage water, but the differences were statistically significant ($p < 0.05$) only at weeks 21 and 22 (Fig. 1).

Changes in DON concentrations

The litter treatments had significant overall effects on DON in drainage water ($F = 14.270$, $p < 0.001$). The temporal trends shown in Fig. 2a demonstrate that the presence of litter enhanced the DON concentrations in the drainage water overall from weeks 2 to 31. However, the two litter treatments showed marked differences in their DON production over time compared to the control treatment; the subsurface litter treatment showed substantial enhancement from weeks 3 to 12 whereas the surface litter showed substantial enhancement during weeks 24–31.

Changes in TDN concentrations

Concentrations of TDN in the drainage water were affected significantly by the application of litter either at surface or below the surface ($F = 17.044$, $p < 0.001$; Fig. 2b). TDN started rising sharply for the control and surface litter treatment from week 1 (early winter) and peaked during week 5 for the control and week 6 for the surface litter treatment; largely this reflects the corresponding NO_3^-–N data in Fig. 1a and b. In contrast, TDN in drainage water from the subsurface litter treatment remained much lower at this time in winter (weeks 3–11) compared with TDN for the control and surface litter treatments, reflecting the lower NO_3^-–N contribution for this treatment. TDN from each treatment was lower in the drainage water from week 12, remaining relatively constant between weeks 14 and 21 but increasing slowly from weeks 23 to 31 (early to mid summer). The latter reflects increases in both DIN and DON over the corresponding period.

The mean of DON as a % of TDN for each treatment increased in the order: control (mean = 57.4%, range = 2.3–99.4%, $n = 90$) < surface litter (mean = 60.8%, range = 5.9–97.7%, $n = 90$) < subsurface litter (mean = 64.7%, range = 17.7–98.6%, $n = 90$). Mean weekly data from the three treatments showed that DON constituted 15–96% of TDN over 31 weeks (data not shown).

Litter effects on drainage water DOC concentrations

Concentrations of DOC showed distinct trends over time from week 1 (early winter) to week 31 (mid summer), with treatments affecting DOC significantly ($F = 32.010$, $p < 0.001$; Fig. 2c). DOC from the subsurface litter treatment remained highest until week 14 compared with DOC for control and subsurface litter treatments; however, the concentrations declined sharply for each treatment until week 4 (Fig. 2c). As for DON, the subsurface litter treatment produced higher DOC in early-late winter (weeks 1–14) but the surface litter treatment produced higher DOC in the drainage water from spring to mid summer (weeks 17–31). DOC for the control treatment was similar to that for the surface litter treatment from weeks 5 to 12, and to that for the subsurface litter treatment from weeks 17 to 31 (Fig. 2c).

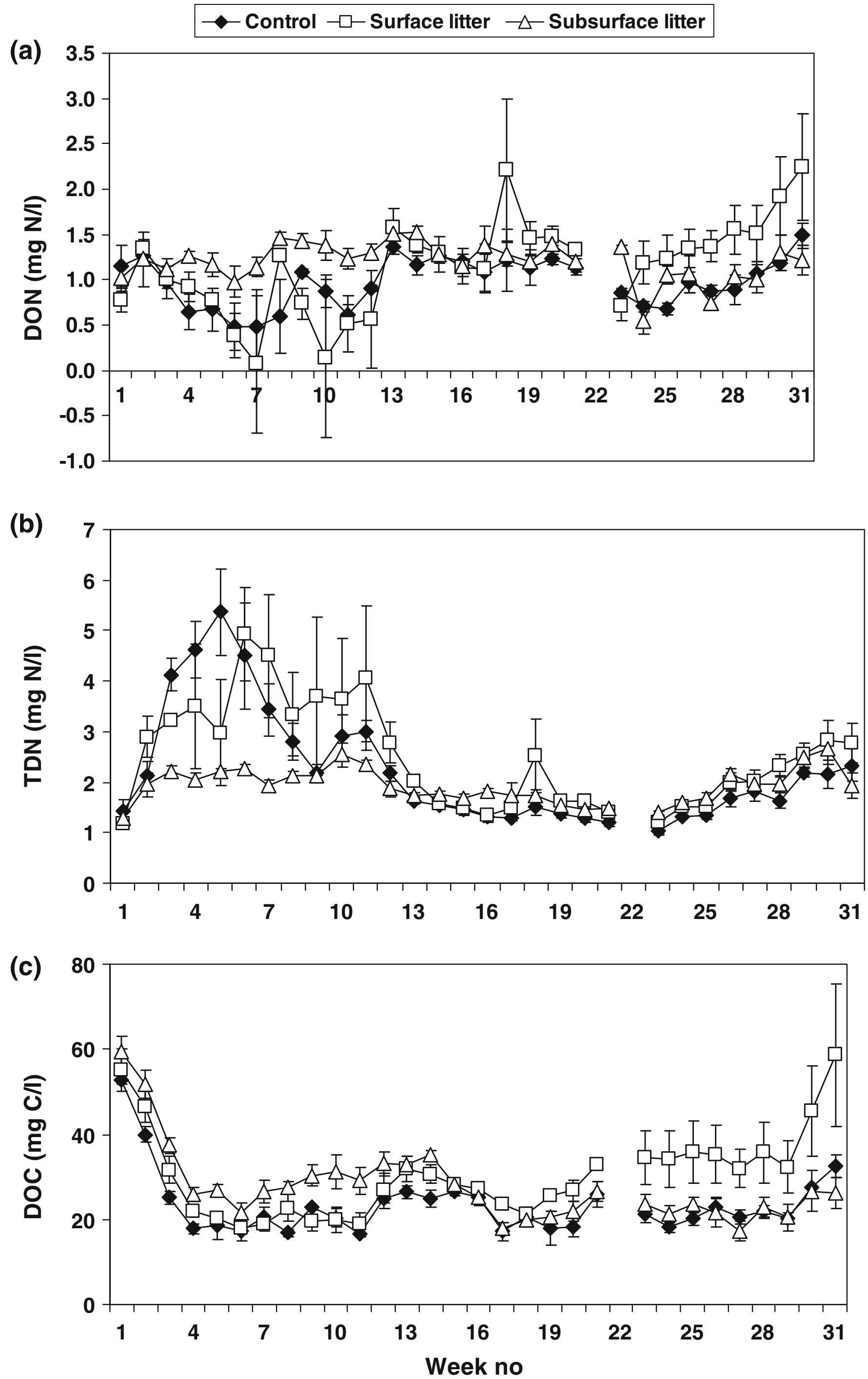

Fig. 2 Concentrations of **a** DON (mg C/l), **b** TDN and **c** DOC (mg N/l) for three treatments over 31-week outdoor incubation study from week 1 (1–8 December 2008, early winter) to week 31 (30 June to 6 July 2009, mid summer).. All values are means of three replicates. *Error bars* are standard error of means. Values were not computed during week 22 due to instrumental failure

Drainage water DIN, DON and DOC fluxes

The NH_4^+–N fluxes remained below 20 mg N/m^2/week (ca. 0.2 kg N/ha/week) for each treatment, but were higher in early winter (weeks 1–7) for the subsurface litter treatment and in mid summer for the surface litter treatment (Fig. 3a). The large flush of NH_4^+–N during the freeze–thaw cycles in weeks 10–12 provided substantial amounts of N leached as NH_4^+–N in drainage water. The NH_4^+–N fluxes

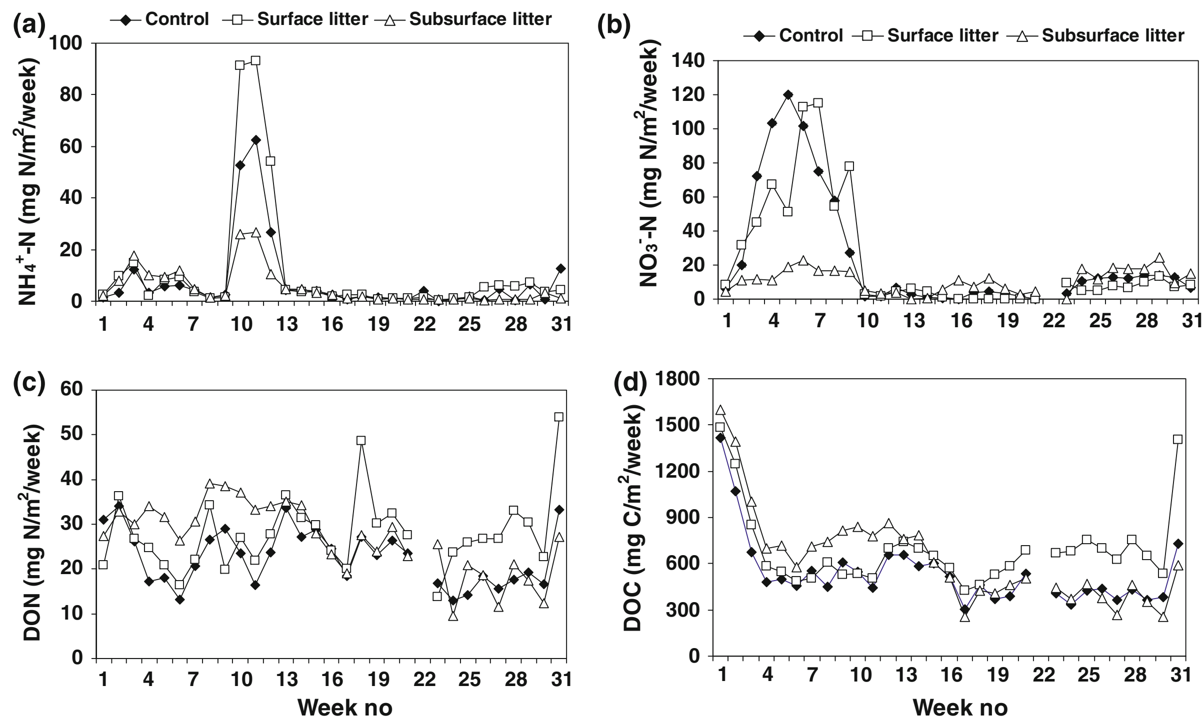

Fig. 3 Weekly fluxes (mg/m^2) of leached **a** NH_4^+–N, **b** NO_3^-–N, **c** DON and **d** DOC from microcosms for the three treatments from week 1 (1–8 December 2008, early winter) to week 31 (30 June to 6 July 2009, mid summer). Values are means of three replicates. Fluxes were not calculated for DON and DOC during week 22 due to instrumental error

during weeks 10–12 accounted 60, 68 and 39% of the total NH_4^+–N flux for the control, surface litter and subsurface litter treatment respectively (from Fig. 3a).

The NO_3^-–N flux in the drainage water was considerably higher from the control and the surface litter treatments compared with the subsurface litter treatment during weeks 1–9 in winter (Fig. 3b). Later, NO_3^-–N fluxes were generally higher for the subsurface litter treatment (during weeks 15–19, 24, 26–29 and 31 in spring to mid summer). There were slight increases in NO_3^-–N fluxes for all treatments during weeks 24–31 in late spring to mid summer. In winter over weeks 1–9, cumulative fluxes of NO_3^-–N in the drainage water for the control treatment (583 mg N/m^2 or 5.83 kg N/ha) and the surface litter treatment (563 mg N/m^2 or 5.63 kg N/ha) were much higher compared with fluxes for the subsurface litter treatment (130 mg N/m^2 or 1.30 kg N/ha) (from Fig. 3b).

DON fluxes fluctuated relatively more over time and generally remained between 10 and 40 mg N/m^2/week for the three treatments, with occasional higher weekly DON fluxes (≥50 mg N/m^2/week) for the surface litter treatment in spring or summer (weeks 18 and 31) (Fig. 3c). However, in winter from weeks 3 to 12 and in week 15, the subsurface litter produced higher fluxes compared with the control and the surface litter treatments. The surface litter treatment resulted in higher DON fluxes from weeks 18 to 21 in spring and then during weeks 24–31 in summer, compared with the control and the subsurface litter treatment (Fig. 3c).

The shape of time series plots for DOC fluxes was very similar to that for DOC concentrations for each treatment (compare Figs. 2c, 3d). Even after the significant reduction in DOC fluxes until week 4 for each treatment, DOC fluxes were still higher for the subsurface treatment from weeks 1 to 14 throughout winter compared with the control treatment and surface litter treatment (Fig. 3d). Subsequently, the surface litter treatment produced relatively higher DOC fluxes from weeks 15 to 31 compared with the control and subsurface litter treatments, which varied from lower values of 253 to 608 mg C/m^2/week. The cumulative DOC flux over weeks 1–3, however, accounted for 20, 17 and 21% of the total DOC flux for the control, surface litter and subsurface litter treatments respectively.

Temperature regulation of drainage water DON and DOC dynamics

When fresh litter was applied, it initially decomposed faster during the early stage of the experiment regardless of the low winter temperature. A surge in decomposer activities led to a flush of DOC, but not DON, as litter can substantially retain N (compared to control soil) at early stages of decomposition (Fig. 2a, c). Consequently, when assessing the long-term temperature impact on DOC and DON, data points from weeks 1 to 3 observations were excluded from data analysis.

Possible temperature dependence of DON and DOC, from weeks 4 to 31, is shown in Figs. 4 and 5 respectively. Responses for each treatment type to changes in ambient temperature were remarkably similar for DON and DOC. Temperature appeared to have a significant positive effect on DON production for the control treatment ($r^2 = 0.31$, $p < 0.01$; Fig. 4a). Similarly, when litter was present at the surface, DON was significantly positively correlated with temperature ($r^2 = 0.56$, $p < 0.001$; Fig. 4b). However, there was non-significant negative correlation between DON and temperature for the subsurface litter treatment ($r^2 = 0.05$, $p = 0.267$; Fig. 4c).

The general increase in temperature from winter to summer apparently had a similar significant positive effect on DOC for the control and the surface litter treatments; the effect, as for DON, was much stronger for the surface litter treatment ($r^2 = 0.75$, $p < 0.001$; Fig. 5b) than for the control treatment ($r^2 = 0.31$, $p < 0.01$; Fig. 5a). However, for the subsurface litter treatment, temperature had weak but significant negative effect on DOC in the drainage water ($r^2 = 0.15$, $p < 0.05$; Fig. 5c).

Figure 6a demonstrates that the increase in temperature apparently resulted in a significant negative correlation between the DOC:DON ratio and temperature for the control treatment only ($r^2 = 0.28$, $p < 0.05$). In contrast, temperature had a significant positive correlation with the DON:DIN ratio (Fig. 6b) for the control treatment ($r^2 = 0.17$, $p < 0.05$) and for the surface litter treatment ($r^2 = 0.29$, $p < 0.01$), but the relationship was non-significant for the subsurface litter treatment.

Relationships of DIN, DON and DOC concentrations with pH, EC, SO_4^{-2} and Cl^-

DIN and DON concentrations in drainage water were significantly negatively ($p < 0.01$) correlated for each treatment (Table 2). DIN and DOC were significantly negatively correlated ($p < 0.01$) for the surface litter treatment only. DOC and DON concentrations showed significant positive ($p < 0.01$) correlations for all treatments.

DIN showed a significant negative ($p < 0.01$) correlation with pH, but DON a significant positive correlation ($p < 0.01$) with pH, for the control treatment. DON was also negatively correlated ($p < 0.05$) with pH for the subsurface litter treatment (Table 3). DON and DOC were both significantly negatively correlated ($p < 0.01$) with EC for the control and surface litter treatments only. Interactions between DOC and Cl^- were significant and negative

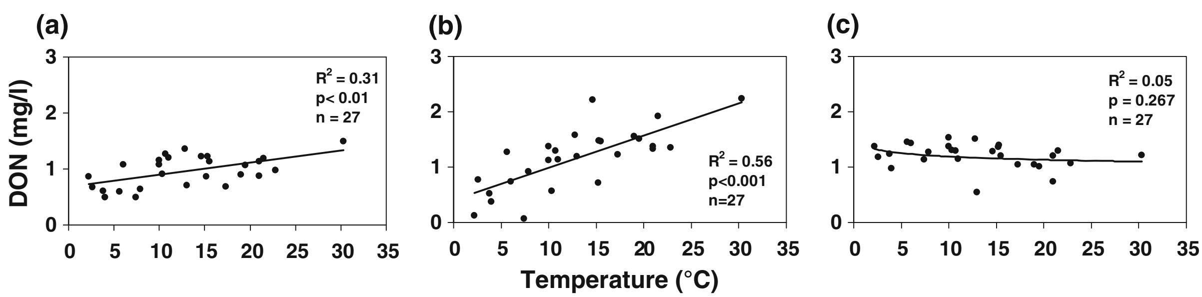

Fig. 4 Effect of mean weekly temperature on drainage water mean weekly DON (mg/l) concentrations of **a** control, **b** surface litter and **c** subsurface litter treatment. The data for each treatment excludes observations from initial 3 weeks

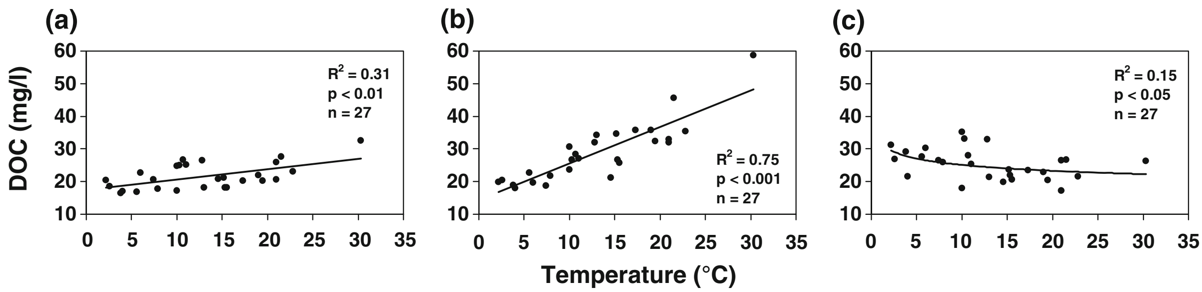

Fig. 5 Effect of mean weekly temperature on drainage water mean weekly DOC (mg/l) concentrations of **a** control, **b** surface litter and **c** subsurface litter treatment. The data for each treatment excludes observations from initial 3 weeks

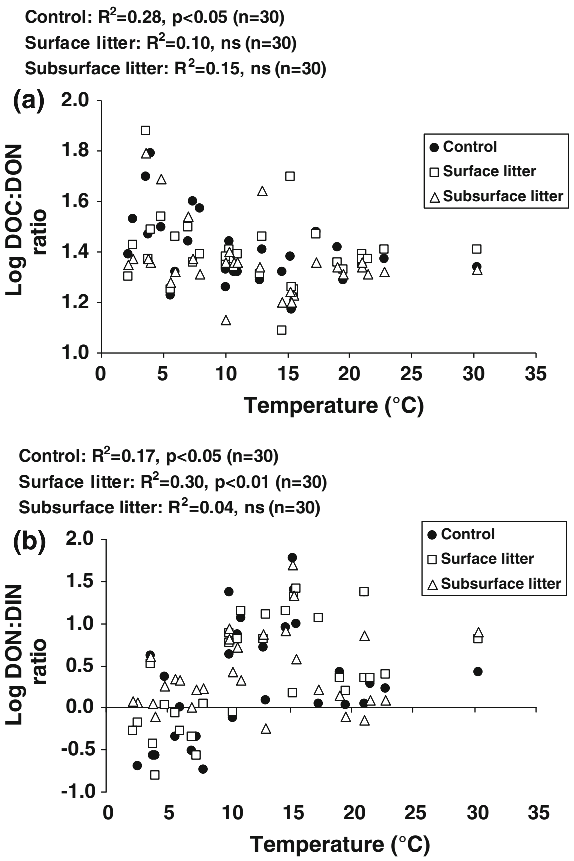

Fig. 6 Effect of the mean weekly temperature on **a** mean weekly DOC:DON ratio and **b** mean weekly DON:DIN ratio for three treatments

($p < 0.01$) for the control and surface litter treatments. Both SO_4^{-2} ($p < 0.01$) and Cl^- ($p < 0.05$) concentrations significantly positively correlated with DON for the subsurface litter treatment (Table 3).

DOC showed negative correlations with NO_3^-–N for the control ($r = -0.259$, $p < 0.05$, $n = 76$), the surface litter ($r = -0.379$, $p < 0.01$, $n = 69$) and the subsurface litter treatments ($r = -0.425$, $p < 0.01$, $n = 84$). In contrast, NH_4^+–N and DOC were correlated positively ($r = 0.441$, $p < 0.01$, $n = 90$) for the subsurface litter treatment but not for the control or surface litter treatments. The concentrations of NH_4^+–N were also positively correlated ($r = 0.243$, $p < 0.05$, $n = 90$) with SO_4^{-2} concentration for the subsurface litter treatment.

Net cumulative N and C flux balances in drainage water

Cumulative input flux for NH_4^+–N was 588 mg N/m^2 and that for NO_3^-–N was 269 mg N/m^2 but zero for DON or DOC (Table 4). There was no significant difference between the treatments for cumulative output NH_4^+–N flux. However, cumulative NO_3^-–N flux in the drainage water was significantly ($p < 0.05$) lower in the subsurface litter treatment compared with the control and the surface litter treatments. Cumulative DIN and TDN outputs were lowest from the sub-surface litter treatment. Cumulative DON fluxes were significantly ($p < 0.05$) higher for litter treatments compared with the control treatment. For litter treatments, the presence of litter compared with the control resulted in higher share of DON in the TDN output fluxes (46% for surface litter, 62% for subsurface litter), and lower shares of NO_3^-–N (from Table 4). However, the contribution of NH_4^+–N in the cumulative TDN output flux was higher for the surface litter treatment (19%) compared with the control (14%) and the subsurface litter treatments (13%) (from Table 4). Cumulative DOC fluxes differed significantly ($p < 0.05$) between the treatments and varied in the order: control < subsurface litter < surface litter (Table 4).

Table 2 Correlation matrix of DIN, DON and DOC concentrations for each treatment

Treatment	Drainage water chemical determinants	DIN	DON	DOC
Control ($n = 90$)	DIN	1		
	DON	−0.533*	1	
	DOC	ns	0.477*	1
Surface litter ($n = 89$)	DIN	1		
	DON	−0.507*	1	
	DOC	−0.389*	0.543*	1
Subsurface litter ($n = 90$)	DIN	1		
	DON	−0.421*	1	
	DOC	ns	0.359*	1

ns non-significant results

* $p < 0.01$ level of significance

Table 3 Correlation coefficients of drainage water pH, EC, DOC, SO_4^{-2} and Cl^- with drainage water DIN, DON and DOC concentrations for each treatment

Treatment	Drainage water chemical determinants	DIN	DON	DOC
Control ($n = 90$)	pH	−0.648**	0.398**	ns
	EC	0.735**	−0.506**	−0.374**
	Cl^-	ns	ns	−0.458**
	SO_4^{-2}	ns	ns	ns
Surface litter ($n = 89$)	pH	−0.475**	ns	ns
	EC	0.674**	−0.302**	−0.419**
	Cl^-	0.275**	ns	−0.396**
	SO_4^{-2}	ns	ns	ns
Subsurface litter ($n = 90$)	pH	−0.432**	0.244*	ns
	EC	0.459**	ns	ns
	Cl^-	ns	0.236*	ns
	SO_4^{-2}	ns	0.339**	ns

ns non-significant results

* $p<0.05$, ** $p < 0.01$ level of significance

Net cumulative fluxes clearly indicated retention of NH_4^+–N without any significant differences between the treatments (Table 4). However, net NO_3^-–N flux was significantly ($p < 0.05$) and substantially lower for the subsurface litter treatment compared with the control and surface litter treatments. Net DIN flux strikingly demonstrated significantly ($p < 0.05$) higher retention of DIN associated with the subsurface litter treatment compared with its counterparts. It is clear from Table 4 that DON dominated the TDN net fluxes for each treatment. Furthermore, cumulative DOC:cumulative DON ratio showed significant negative correlations with cumulative NO_3^-–N flux ($r^2 = 0.99$, $p < 0.01$) and cumulative DIN flux ($r^2 = 0.97$, $p < 0.05$) in the drainage water (Fig. 7), although these should be viewed with caution because of the non-linear data distribution.

Discussion

Litter effects on DIN concentrations and fluxes

Litter effects on DIN concentrations in the drainage water in winter differed substantially depending on whether it was applied on or below the surface. However, there were no convincing differences in DIN concentration in drainage water between the control and surface litter treatments. DIN in the drainage water, particularly from the control and surface litter treatments, was dominated by NO_3^-–N in winter until week 9, though differences between the treatments were significant only in weeks 3, 7 and 8. The mobility of NO_3^-–N into surface and groundwaters is a frequently observed phenomenon, especially in winter when biological N uptake is

Table 4 Cumulative N input–output budget (mg N/m^2) and cumulative amount of C (mg C/m^2) leached in drainage water over the 31 weeks duration

Treatment	Input in simulated rain			Output in drainage water						Net balance[a]			Net TDN balance in drainage water
	NH_4^+–N	NO_3^-–N	DIN	NH_4^+–N	NO_3^-–N	DIN	DON	TDN	DOC	NH_4^+–N	NO_3^-–N	DIN	
Control	588	269	857	231[a]	710[a]	941[a]	678[a]	1601[ab]	16156[a]	(−) 357[a]	(+) 441[a]	(+) 84[a]	744[ab]
Surface litter	588	269	857	348[a]	657[a]	1005[a]	836[b]	1782[a]	20703[b]	(−) 240[a]	(+) 388[a]	(+) 148[a]	925[a]
Subsurface litter	588	269	857	161[a]	325[b]	486[b]	802[b]	1278[b]	19037[c]	(−) 427[a]	(+) 56[b]	(−) 371[b]	421[b]

Weekly fluxes from each of three replicate drainage samples for each treatment over 31 weeks were summed and used to test for significance differences between treatments by ANOVA and Tukey's HSD post hoc test

Values in each column sharing different letters differ significantly from each other at $p < 0.05$

[a] (−) net retention and (+) net loss

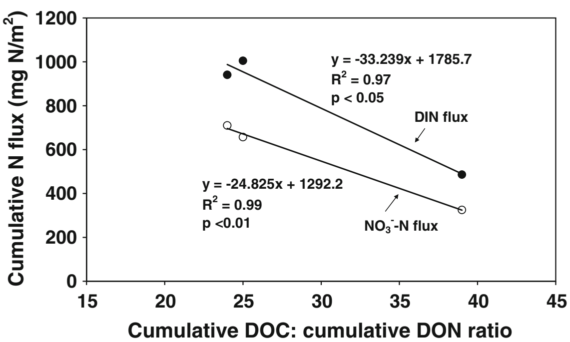

Fig. 7 Relationship of cumulative DOC:cumulative DON ratio with NO_3^-–N and DIN flux in the drainage water. The values were computed from Table 4 to draw the scatter plot

reduced many fold (Chapman and Edwards 1999). The peaking of NO_3^-–N in drainage water in winter, particularly for the control and surface litter treatments, is in line with many studies (e.g. Likens et al. 1970; Skinner et al. 1997). Similar trends have been found in the Lake District, UK (Sutcliffe et al. 1982), in upland moorland catchments (Chapman 1994) and in the River Derwent, N. Yorkshire, UK (Mian et al. 2010). But the presence of litter as the subsurface treatment remarkably reduced the NO_3^-–N in the drainage water in winter (significant in weeks 3, 7 and 8 only), suggesting enhanced NO_3^-–N retention probably by microbial immobilization. This may be attributed to the C:N ratio of incorporated litter (17.7) exceeding that of the subsoils (13.4). This could potentially immobilize the available NO_3^-–N and reduce its mobility in drainage water (Christ et al. 2002). Hefting et al. (2005) also noticed that decaying leaf litter resulted in small, but significant amounts, of NO_3^-–N immobilization in winter in riparian zones they studied. It is also noticeable that subsurface litter pre-dominantly immobilizes NO_3^-–N as NO_3^-–N concentrations were greatly reduced by this treatment but differences between the treatments for NH_4^+–N concentrations were not significant except in week 4.

Appreciable amounts of NH_4^+–N occurred in the drainage water from each treatment throughout the experiment but with distinct temporal variations. This is perhaps surprising as NH_4^+–N mobility usually is largely ignored (Fernando et al. 2005). Very recently Mian et al. (2009) and Lorz et al. (2010) have proved that NH_4^+–N can contribute significantly towards DIN in soil leachates. We observed very conspicuous freeze–thaw cycle effects on NH_4^+–N concentrations which resulted in large flushes of NH_4^+–N in the drainage water during weeks 10–13; this effect was greatest for the surface litter treatment, followed by the control and subsurface litter treatments. It is probably due to increased N mineralization after soil freezing (Edwards and Cresser 1992) as microbes or enzymes react with amino acids released from killed cells. Hinman (1970) studied the impact of repeated freeze–thaw events on some Canadian soils and also found increased extractable NH_4^+–N, while NO_3^-–N concentrations remained unaffected.

Taking into account the DIN input concentrations (1.02 mg N/l) in the simulated rain, clearly litter layers and organic matter-rich surface soil layers were the source of additional DIN in the drainage water in winter, probably via decomposition of fresh organic matter. Bryant et al. (1998) found higher N release from plant litter at early stages of decomposition. On the other hand, decaying litter in the subsurface litter treatment retained most of the DIN in winter. DIN concentrations increased gradually in the late summer with the increase in temperature. Williams and Anderson (1999) found substantial amounts of NH_4^+–N produced in the litter layer and potentially moved into lower mineral soils layers. Riaz et al. (2008) also suggested that the drainage water DIN concentrations represent the net effects of N mineralization and immobilization in soil profiles. Gradual increases in DIN concentrations in summer with the increase in temperature may simply reflect temperature effects which enhance the organic matter mineralization, as described by Sierra (1997).

Fluxes of DIN followed similar trends and patterns to those for DIN concentrations with respect to treatment effects and temporal variations. NH_4^+–N fluxes leached during the freeze–thaw cycles contributed substantially (>60%) towards the cumulative flux over 31 weeks, especially for the control and surface litter treatments, but much less (<40%) for the subsurface litter treatment which strongly suggests that the subsurface litter treatment moderated the effect of freeze–thaw cycles. Similarly, for NO_3^-–N flux, the subsurface litter treatment retained NO_3^-–N much more efficiently in winter, when NO_3^-–N leaching could potentially occur to degrade water quality.

Litter effects on DOC and DON dynamics

Litter addition, both as surface and subsurface treatments, enhanced the DOC in the drainage water. The sharp decline in DOC until week 4 could be attributable to an initial flush of DOC from decomposition of freshly added litter (Fröberg et al. 2007). It may partly be due the fact that soils and litter were re-packed to construct microcosms, hence disturbed from natural conditions which could result in the system being much more dynamic and organic matter turning over relatively faster. After the first 3 weeks, when the initial DOC flush was over, litter presence, either as surface and/or subsurface treatments, largely produced higher DOC concentrations compared with the control throughout the experiment. Müller et al. (2009) noted substantial DOC generation from litter layers in various litter manipulation studies. Don and Kalbitz (2005) also suggested that litter inputs are the main source of DOC and regarded litter decomposition as the driving factor for DOC dynamics. However, considerably higher DOC generation in summer may indicate that partially humified organic matter was contributing to that DOC, when the DOC source from fresh litter would have diminished (Michalzik et al. 2003). DOC fell, even for the control treatment, over the first 3 weeks which may indicate that partially decayed litter naturally present, along with some fresh litter in the mineral soils, was contributing to DOC generation for this particular treatment. It should be considered, however, that DOC in the drainage water is the net result of its production in the litter and/or top soil layers and retention in the underlying mineral soil layers as reduction in DOC with depth gradient is frequently reported in the literature; the decrease is primarily thought to be related to retention via sorption to soil particles rather than degradation (e.g. reviewed by Kalbitz et al. 2000).

Unlike DOC concentrations, DON concentrations showed no signs of any initial flush, which may reflect that litter layers were acting as a N sink during early stages of litter decomposition (Berg and Cortina 1995). However, the DON concentrations in drainage water remained higher in early winter to mid summer for litter-treated microcosms. Park and Matzner (2006) found a small increase in DON concentrations after application of fresh litter. While discussing the origins of DON found in stream water, Campbell et al. (2000) listed various sources, including throughfall, leaching, and decomposition of litter and SOM. DON sinks include adsorption to mineral and organic particles and removal by plant and microbial uptake; however, the latter is considered much less important (Finlay et al. 1992; Northup et al. 1995). However, Gregorich et al. (2003) found 50% DON mineralization in less than 10 days. The DON concentrations in drainage water from each treatment should be interpreted as the net results of these processes.

Seasonal variations and apparent effects of temperature

After the initial flush of DOC from fresh litter (Don and Kalbitz 2005), DOC concentrations consistently increased for the surface litter treatment and were highly correlated with weekly mean noon temperature. A similar, but weaker, relationship was found for the control treatment. A number of studies have shown the highest DOC concentrations in leachates during late summer (e.g. Kaiser et al. 2002; Kalbitz et al. 2000; Yano et al. 2000). Increased DOC in summer is largely attributed to temperature-dependent enhanced biological activity accelerating litter decomposition (McDowell and Likens 1988). The weak, but significant, negative correlation found between DOC concentrations and weekly mean noon temperature for the subsurface litter treatment may suggest enhanced decomposition of mobile organic matter, thereby reducing DOC concentrations throughout the soil profile with increase in temperature, or possibly over time.

As for DOC, concentrations of DON were slightly higher in summer for the surface litter treatment compared with the control, and significantly positively correlated with temperature. This increase in DON in late summer may also be attributed to increased temperature which generally triggers decomposers activity (Hedin et al. 1995). However, the significant negative temperature effect on DON for the subsurface litter treatment may indicate that increase in temperature-dependent consumptive processes outweigh any effect on productive processes. As for DOC though, this effect on DON could partially at least represent changes over time, not necessarily associated with rising temperature. Unequivocal interpretation would require substantial additional parallel experiments conducted under controlled-temperature conditions using a range of temperatures.

Temperature effects on DOC:DON and DON:DIN ratios

The DOC:DON ratio of drainage water correlated negatively and significantly with increase in temperature only for the control treatment. In contrast, DON:DIN ratio was significantly enhanced with increase in temperature for control and surface litter treatments, probably reflecting the high DIN concentrations over winter months. Although some consistent trends in the correlations between DOC:DON ratio and temperature were found, it must be remembered that temperature was increasing over time. It is possible therefore that the relationship for the control primarily reflects the decline in C:N ratio in the absence of fresh litter inputs as mineral N in simulated rain is added over the duration of the experiment. The decline in DOC:DON ratio could therefore simply be reflecting what is happening to SOM in solid phase. The apparent effect of temperature on DON:DIN ratio was not significant for the subsurface litter treatment. This difference from the control and surface litter treatment shows the far greater NO_3^-–N retention in the subsurface litter treatment over the winter months. Thus litter incorporation in the field should be expected to alter the relative importance of DON and DIN, as well as retaining NO_3^-–N over winter months.

Inter-relationships of DIN, DON and DOC concentrations

DOC and DON were positively associated for each treatment which suggests tight C and N cycling. Solinger et al. (2001) commented that DON generation was strongly coupled with that of DOC, which indicates similar origin, mobility and retention mechanisms. However, a significant negative correlation between DIN and DON concentrations may indicate low biodegradability and bioavailability of DON which could convert DON into DIN.

The significant negative correlations between NO_3^-–N and DOC for the subsurface litter treatment ($r = -0.425$, $p < 0.01$) and surface litter treatment ($r = -0.379$, $p < 0.01$) were stronger than that for the control treatment ($r = -0.259$, $p < 0.05$). This may indicate a potential role for DOC in NO_3^-–N immobilization or that mineralization of DOM produces NH_4^+–N that is readily nitrified. Goodale et al. (2005) suggested microbial NO_3^-–N retention stimulated by increased DOC concentrations and proposed this as the potential explanation for reduced NO_3^-–N export to a number of streams in New Hampshire in mid 1990s. This may well explain reduced NO_3^-–N concentrations associated with the subsurface litter treatment.

The significant positive association of NH_4^+–N with DOC for the subsurface litter treatment indicates a possible role of DOC in NH_4^+–N mobility, or that both were generated simultaneously. This could support the idea of ready mineralization of DOM to produce NH_4^+–N, only some of which is nitrified. Stevenson (1994) proposed formation of DOC–NH_4^+–N complexes particularly when their concentrations were high which could lead to significant NH_4^+–N export into the drainage water. However, the positive relationship between NH_4^+–N and DOC contradicts findings of Magil and Aber (2000) who found less DOC release in the presence of added NH_4^+–N primarily because of enhanced DOC metabolism in the presence of the more readily available form of N.

The potential role of mobile mineral acid anions in NH_4^+–N mobility may also be considered in the context of DIN dynamics in the drainage water. Significant positive associations were found between NH_4^+–N and SO_4^{-2} concentrations for the subsurface litter treatment only. Duckworth and Cresser (1991) found increased NH_4^+–N mobility from litter horizons in the presence of sea salts.

Relationships of DON and DOC concentrations with pH and EC

The significant negative correlation of DON and DOC with EC for the control and surface litter treatment agrees with many reported studies indicating that increase in ionic strength of solution decreases DOC mobility (Kalbitz et al. 2000). No such correlation was not found for the subsurface litter treatment which suggests a moderation effect if litter is incorporated into subsurface soil layers. Similarly, the significant positive correlation between DON and pH for the control and subsurface litter treatments supports many laboratory observations (e.g. Vance and David 1989; Andersson et al. 2000). However, lack of a similar correlation for the surface litter treatment contradicts the findings summarized by Kalbitz et al. (2000). No correlation was found between DOC and pH for any of the treatments. Nieder and Benbi (2008) discussed the various studies indicating inconsistent relationships between pH and DOC; some showed positive associations while others showed no relationship. Examination of time-series plots for EC, SO_4^{-2} and Cl^- (data not shown) clearly indicated substantial release of SO_4^{-2} and Cl^- in the early winter period, especially from the surface litter treatment. This reflects closely the strong temporal trends for NO_3^-–N seen in Fig. 1, suggesting that the correlations in the Table 3 are probably more co-incidental than causal.

Cumulative N and C fluxes and net budgets

Cumulative fluxes of DIN, DON and DOC showed clear litter effects on N and C dynamics in this relatively short-term experiment. Significantly lower NO_3^-–N and DIN fluxes in drainage water strongly demonstrate reduction in mineral N losses when litter was manipulated into subsoils, even though the litter C:N ratio was only 17.7. This was however appreciably higher than the C:N ratio of soils at 10–25 cm depth. DON fluxes were significantly higher for the litter treatments compared with the control treatment. This suggests that litter is the major source of DON generation as the simulated rain lacked DON. DOC fluxes were significantly higher for the litter treatments and differed significantly between the treatments, in line many studies showing litter as well as humified organic matter contributing to DOC generation (e.g. Currie et al. 1996; Kalbitz et al. 2000; Neff and Asner 2001). The subsurface litter treatment significantly reduced cumulative TDN flux compared with the control and also markedly changed the N species composition, resulting in DON dominance over DIN in the cumulative net TDN output flux.

Net balance (output–input flux) showed that each treatment, on balance, retained NH_4^+–N equivalent to most of the NH_4^+–N applied in the simulated rain while the positive net fluxes for NO_3^-–N showed that some of NH_4^+–N from the simulated rain could be nitrified adding to NO_3^-–N from organic matter mineralization; no treatment effects were significant however. Adamson et al. (1993) found substantial NH_4^+–N production in organic matter-rich soil layers and its complete retention in subsequent mineral soil layers. However, net NO_3^-–N balance shows extra NO_3^-–N production within the soil profiles for the control and surface litter treatment compared with the subsurface litter treatment. An alternative way of interpreting this is that the subsurface litter treatment remarkably and significantly reduced NO_3^-–N and DIN net fluxes. This could be linked to C:N ratio of added litter which has been shown to immobilize N and restrict its export primarily as NO_3^-–N (Idol et al. 2003). The output of DON in drainage water constituted more than 90% of the TDN flux for the control and surface litter treatments. Studies describing the proportion of TDN lost as DON from grasslands usually imply lower proportional loss. For example, Hawkins et al. (1997) found 20% of the total N lost was DON in a grassland ecosystem in Devon, UK. However, such studies are not considering net fluxes. The role of subsurface litter was strikingly different in this context, as it not only retained most of the DIN but also almost doubled the

DON to DIN ratio compared with that for the surface litter treatment.

Campbell et al. (2000) suggested that the use of stream water C:N ratio, i.e. DOC:DON ratio could be an effective predictor for N leaching and N status of sites. They perceived DOC and DON measurements in stream water as being advantageous over the soil C:N ratios as they are easier to measure, less prone to spatial variability and provide reliable assessment for the whole watershed. Thus drainage water C:N ratios could serve as useful evaluation parameter for N dynamics and C controls on them. As supportive evidence, they found a significant negative relationship between annual DIN flux and stream water DOC:DON ratio. The results of the current study support this as strong negative linear relationships were found between drainage water cumulative DOC:cumulative DON ratio and NO_3^-–N and DIN fluxes, which indicates the importance of litter inputs on N mobility in such acid grassland soils. They also highlight the potential of using this relationship as an indicator of the N status of the ecosystem as, over the entire current study period, the subsurface litter treatment resulted in higher DOC:DON ratio and markedly reduced the total NO_3^-–N and DIN export in the drainage water.

How useful is it to measure weekly fluxes and design a short-term study?

Concentrations and fluxes were measured weekly in the drainage water in the present study. This is not common as researchers mostly look at monthly and seasonal trends in N species concentrations and fluxes. Quantifying weekly fluxes was helpful in detecting important episodic effects like the flush of DOC from freshly added litter (weeks 1–3) and of NH_4^+–N from freeze–thaw cycles (weeks 10–12). Data interpretation highlighted the importance of these two events in quantification of C and N fluxes. It was possible only because the experimental design involved weekly measurements, as otherwise the scale of these pulses of potentially high ecological significance would have been missed. Although the current study was relatively short-term, monitoring drainage water chemistry may indicate long-term changes which can be induced by litter manipulation in soils, as suggested by McDowell (2003).

Conclusions

This reconstituted microcosm study, using freely draining sandy acidic grassland soil from Hob Moor, York, was conducted at outdoor ambient temperatures for 31 weeks, from early winter to mid summer. It showed:

- Litter manipulation altered the DIN dynamics and litter incorporation to 15 cm remarkably reduced NO_3^-–N concentrations in the drainage water in winter when NO_3^-–N is otherwise more likely to be leached due to reduced biological uptake.
- NH_4^+–N was measureable in drainage water from each treatment. Freeze–thaw cycles increased NH_4^+–N mobility, but less markedly for the subsurface litter treatment.
- DOC showed a significant negative correlation with NO_3^-–N concentration for each treatment suggesting either a role for DOC in reducing NO_3^-–N export in drainage water as DOC may enhance microbial retention of NO_3^-–N or that DOM mineralization favours enhanced NO_3^-–N production.
- DOC and DON concentrations in drainage water both correlated positively with temperature, but the subsurface litter treatment moderated the temperature effect. However, this cannot be attributed unequivocally to increasing temperature, because factors other than temperature changed over time in this experiment.
- Significant differences between treatments for cumulative DOC and DON fluxes suggested that litter was the primary source of their generation.
- Net cumulative TDN fluxes suggested complete DIN and partial DON retention associated with the subsurface litter treatment.
- Cumulative DOC:cumulative DON ratio showed significant negative correlation with cumulative NO_3^-–N and DIN fluxes, indicating its potential use as effective predictor for DIN leaching.

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