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REGULAR ARTICLE

Nitrogen turnover in fresh Douglas fir litter directly after additions of moisture and inorganic nitrogen

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Abstract The effects of wetting and drying and
inorganic nitrogen (N) addition on carbon (C) and N
turnover in fresh Douglas fir litter (Speuld forest, the
Netherlands) were investigated. Litter was incubated
for 9 days in the laboratory, receiving different
moisture and N addition treatments. Following the
additions, a series of reactions were observed of
which most notable were a rapid retention of added
ammonium and nitrate (NO_3^-) and a sudden increase
in CO_2 respiration. For the rewetted-and-moist incu-
bations, respiration levels remained elevated, N was
net immobilized and nitrous oxide (N_2O) production
increased throughout the experiment. About 80% of
the NO_3^- produced was lost again as N_2O. In the
rewetted-and-dried incubations, respiration decreased
during the drying phase; no clear patterns in N
mineralization were detected; and N_2O production
remained at constant levels, but still resulted in
gaseous loss for half of the NO_3^- net produced. The
experiments thus revealed two important NO_3^- sinks
in LF1 litter, namely rapid retention of added NO_3^-
and gaseous loss as N_2O. The maximum NO_3^- loss
via these sinks was estimated at 2 kg-N ha^{-1} yr^{-1},
which is small compared to annual NO_3^- leaching at
90 cm soil depth (31 kg-N ha^{-1} yr^{-1}).

Keywords Douglas fir · Litter · Drying and rewetting ·
Nitrogen transformations · Respiration · Nitrous oxide

Introduction

Forests in the Netherlands have experienced elevated
atmospheric N inputs for nearly 50 years now, and the
growing concern about the effects of these increased
inputs on forest ecosystem functioning has initiated
intensive scientific research in the field of N cycling
since the early 1980s. In general, the studies under-
taken have a temporal resolution of weeks to years,
either by direct choice (for example to elucidate long-
term changes in the functioning of forest ecosystems
like Koopmans et al. 1996), or because changes in N
pool sizes are slow and require time to be measured
(for example field incubations like in Tietema 1993).
Microbial processes, however, are highly dynamical
and respond rapidly to changes in conditions in the
forest soil, like temperature, moisture and nutrient
inputs. Consequently, while standard low-resolution
N research is important and necessary, it may
overlook mechanisms and processes operating at a
shorter time scale.

In recent years, there has been a growing interest in short-term N dynamics, especially in reaction to moderate treatments of the soil like recursive freezing and thawing, wetting and drying, or fertilizer additions (Kuzyakov et al. 2000). An important driver for this research is climate change that will likely have implications for nutrient availability and leaching losses in soils (Borken and Matzner 2008). For example, Pulleman and Tietema (1999) showed that in the Douglas fir forest of Speuld, the Netherlands, rewetting of dried litter could induce a large, temporary increase in microbial activity and a subsequent temporary net N mineralization flush. Litter from Harvard Forest in northeastern United States has been shown to rapidly immobilize added nitrate (NO_3^-) (Berntson and Aber 2000; Dail et al. 2001), even though this experimental forest had been subject to chronic NO_3^- additions for 11 years. Kuzyakov et al. (2000) summarize a number of possible reactions to inorganic-N additions, including increased N immobilization by microorganisms and acceleration of organic matter decomposition.

In an earlier study (Raat et al. 2002), it was shown that during a rain event not only the forest floor is rewetted, but that simultaneously a large pulse of inorganic-N is added via throughfall. Recorded N pulses were as high as 2.7 kg-N ha^{-1}, equaling about 6.5% of the yearly atmospheric N deposition. Pulleman and Tietema (1999) already emphasized the dynamical reaction of N transformations to rewetting of Speuld litter. Analogous, we hypothesize that inorganic-N addition may also enforce short-term changes in the turnover of C and N in Speuld litter, for example like those put forward by Berntson and Aber (2000) and Kuzyakov et al. (2000).

The aim of the current paper was to gain insight in the effects of simultaneous rewetting and throughfall N additions on the carbon (C) and N turnover in a Douglas fir forest. Focus was on the top LF1 horizon of the forest floor as the first contact of throughfall water is within this horizon. Field moisture conditions just before and directly after a rain event were mimicked in a laboratory incubation experiment, while solutions containing NH_4^- and/or NO_3^- were added. Measurements were conducted on a high frequency basis, i.e. every one to 2 days, to capture possible dynamic responses of C and N turnover to the imposed treatments.

Materials and methods

Site description and sampling

The Speuld research site is a 2.5 ha Douglas fir (*Pseudotsuga menziesii*, Franco L.) forest stand, located in the centre of The Netherlands. The forest was planted in 1962, with an initial tree density of 780 trees ha^{-1}, and was thinned in the winter of 1995–1996 leaving about two-thirds of the trees standing. Understorey vegetation was absent both before and after thinning. The forest floor has a thickness of about 6 cm (Schaap et al. 1997) and consists of a 4.5 cm thick F2 horizon, overlain by 1.5 cm of LF1 horizon; an H horizon is mostly absent. The humus form is classified as a Mormoder (Green et al. 1993). The soil is a well-drained Haplic Podzol (FAO 2006) with a groundwater level at 40 m depth throughout the year. Average precipitation is 834 mm yr^{-1}, while mean potential evapotranspiration is 712 mm yr^{-1} (Tiktak and Bouten 1994). Mean January temperature is 1.5°C; July temperature is 17.0°C (Bosveld et al. 1993). The forest is considered nitrogen saturated, as a result of nearly 50 years of elevated atmospheric N input (Koopmans et al. 1996; Van Breemen and Verstraten 1991). Nitrogen deposition in throughfall amounts to 42 kg-N ha^{-1} yr^{-1}, mainly in the form of NH_4^+. Nitrogen leaching at 90 cm soil depth is 31 kg-N ha^{-1} yr^{-1}, mainly as NO_3^-. Nitrogen transformations occur mainly in the forest floor (Tietema et al. 1993).

The Speuld Douglas fir forest has been subject of intensive research since the mid-1980s. Relevant work with respect to the current study include investigations of N mineralization and nitrification (Tietema et al. 1993; Tietema and Wessel 1992), anoxic micro-sites in litter in relation to N_2O production (Van der Lee et al. 1999), C and N transformations during drying and rewetting (Pulleman and Tietema 1999) and forest floor hydrology (Schaap et al. 1997).

A composite litter sample (LF1 horizon) was obtained in late-autumn by sampling the top 1.5 cm of the forest floor in three 1 m^2 patches, randomly chosen across the forest stand. Cones, branches and roots were removed immediately after sampling. The remaining material was sieved through a 10 mm nylon mesh, air-dried (gravimetric water content $w=$ 115%) and stored dark and cool (2°C) until the

beginning of the experiment. Some chemical characteristics of the litter are given in Table 1.

Experimental set up

Polypropylene jars were filled with the equivalent of 6.0 g dry litter (w=115%) and stored in a climate chamber 2 days prior to the start of the experiment, in order to enable the microbial community to adapt to the incubation temperature of 20°C. Jars were divided into eight series receiving different treatments (Table 2). These treatments involved rewetted-and-continuously-moist (MO) and rewetted-and-dried (WD) incubations, and additions of throughfall solutions containing NH_4^+, NO_3^-, both NH_4^+ and NO_3^-, or no nitrogen at all. Parallel to the samples kept in polypropylene jars we had 8 series in glass jars. These series received the same moisture and N addition treatments and were used to determine CO_2 and N_2O production.

The moisture dynamics imposed to the WD treatments were copied from dynamics in forest floor water content of the F2 horizon of the Speuld Forest as described by Schaap et al. (1997). A single summer rain shower was modeled followed by a 9 day drying period. Drying in our experiment (w=50% after 9 days), however, was somewhat more severe than that observed by Schaap et al. (1997), since we believe that drying is more severe in top LF1 horizon than in the F2 horizon that they studied.

Amounts of NH_4^+ and/or NO_3^- added (Table 2) were calculated from the amounts of nitrogen added to the forest floor during a rain event (2.7 kg-N ha^{-1}; Hansen et al. 1994; Raat et al. 2002), the molar ratio of NH_4^+:NO_3^- in throughfall (2.7:1; Draaijers et al. 1998) and the total dry mass of the forest floor (3.2 kg m^{-2}; Wessel and Tietema 1995). In this calculation we assumed that throughfall was distributed evenly over the top half of the forest floor. In addition to nitrogen species, all added solutions contained a combination of other ions to mimic throughfall of the Speuld forest (Draaijers et al. 1998). These other ions were also added to the control series that lacked N additions. Added concentrations were 1.22, 0.04, 0.20, 0.16 and 0.47 mmol kg^{-1} dry litter for SO_4^{2-}, ortho-P, Ca^{2+}, Mg^{2+} and K^+, respectively. Na^+ replaced NH_4^+ for treatments where no NH_4^+ was added, Cl^- replaced NO_3^-. Added concentrations of Na^+ thus varied between 0 and 2.91, added Cl^- varied between 0.61 and 1.70 mmol kg^{-1} dry litter.

The experiment was started (t=0 days) by dripping concentrated throughfall solutions on the incubated litter using a 1,000 µl pipette. Subsequently, using a conventional plant sprayer, demineralized water was sprayed on the litter in order to spread the added substances equally over the litter and to reach a gravimetric water content of 275%. The MO series were kept in semi-open containers with wet towels. The wet towels created a damp atmosphere that successfully prevented drying of the litter, while the semi-open container still enabled aeration. The WD series were left completely open for the first 3 days to induce air-drying. At t=3.0 days, these series were placed in semi-open containers (but without the wet towels) to somewhat slow down drying. Figure 1 shows the moisture conditions for MO and WD incubations during the course of the experiment.

To characterize the litter's initial conditions, at t= 0.0 days, five samples that had not received any treatment since incubation 2 days prior, were analyzed for NH_4^+, NO_2^- and NO_3^-. First regular sampling started at t=0.13 days, when three randomly chosen samples (n=3) were taken from every serie and destructively analyzed for NH_4^+, NO_2^- and NO_3^-. CO_2 and N_2O production were measured from samples stored in the glass jars (n=3), upon which these were returned to the climate chamber. This sampling procedure was repeated at t=1.0, 2.0, 3.0, 5.0, 7.0 and 9.0 days, but CO_2 respiration and N_2O production were not determined on t=5.0 days. Concentrations of NH_4^+, NO_2^- and NO_3^- were determined colorimetrically in 1.0 M KCl extracts (soil:solution=1:30) on a Skalar continuous flow auto-analyzer. NO_2^- concentrations were always under the detection limit, and so NO_2^- was not used in any further calculations or analysis. CO_2 and N_2O

Table 1 Characteristics of sampled fresh, LF1 horizon Douglas fir litter

Horizon	LF1
Thickness	1.5 cm
pH (1.0 M KCl)	4.3
pH (H_2O)	5.1
C:N	24.7 g g^{-1}
NH_4^+(KCl-extractable)	33.3 mmol-N kg^{-1}
NO_3^-(KCl-extractable)	0.45 mmol-N kg^{-1}

Table 2 Experimental setup. Short names for treatments (as used in the text), imposed moisture conditions and added NH_4^+ and NO_3^- loads

ShortName	Moisture condition	added NH_4^+[mmol-N kg^{-1} dry]	added NO_3^-[mmol-N kg^{-1} dry]
MO-noN	Continuously moist	0.0	0.0
MO-NH$_4$NO$_3$	Continuously moist	2.9	1.1
MO-NH$_4$	Continuously moist	2.9	0.0
MO-NO$_3$	Continuously moist	0.0	1.1
WD-noN	Rewetted and dried	0.0	0.0
WD-NH$_4$NO$_3$	Rewetted and dried	2.9	1.1
WD-NH$_4$	Rewetted and dried	2.9	0.0
WD-NO$_3$	Rewetted and dried	0.0	1.1

production were determined as headspace accumulation of both gasses after airtight sealing of the glass jars. CO_2 was measured on a Carlo Erba GC equipped with a thermal conductivity detector, N_2O on a Varian 3,600 GC equipped with an electron capture detector.

Throughout this paper, concentrations and rates are expressed per kilogram dry weight litter. Time (t) is expressed in days since the addition of the throughfall solutions. N fluxes and rates were determined for 7 time intervals, which are referred to by Roman numbers *i* to *vii* (Fig. 1).

Calculation of N fluxes and statistics

Cumulative N_2O production was estimated by integrating N_2O production rate over time. Net N mineralization rates were estimated from the change in total inorganic-N between two sampling dates. Net nitrification rates were estimated from the change in NO_3^-. Estimates of both net N mineralization and net nitrification were corrected for the loss of NO_3^- as N_2O using the cumulative N_2O production. In addition, estimates of net N mineralization and net nitrification

between $t=0.0$ and $t=0.13$ (time interval *i*) were corrected for the NH_4^+ and/or NO_3^- added. The Student's *T*-test was used to compare means. Differences at the $p=0.05$ level were considered significant.

Results

C and N fluxes directly after remoistening and N additions (time interval i)

As first regular sampling took place only 3 h ($t=0.13$) after the additions, the WD series had not yet lost substantial amounts of water, and moisture contents were the same ($w=275\%$) for both MO and WD series. As such, at this sampling time, both series are treated as one ($n=6$), and the four different N addition treatments are referred to as T-noN, T-NH$_4$NO$_3$, T-NH$_4$ and T-NO$_3$, respectively.

Even though breakdown of fresh litter is often thought to be N limited, prior to the start of the experiments KCl-extractable NH_4^+ concentrations were already high in the LF1 material. Initial NH_4^+
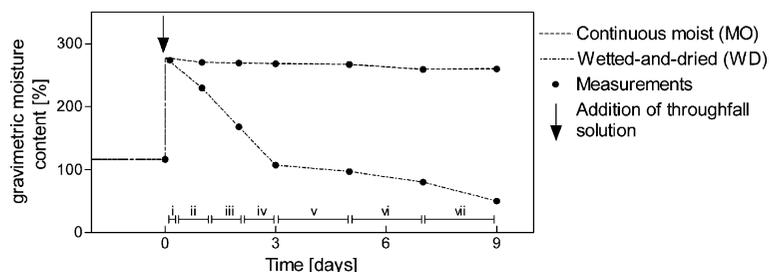
Fig. 1 Gravimetric water content [%] for moist (*MO*) and rewetted-and-dried (*WD*) incubations. Arrow marks time of moisture and N additions, dots mark time of sampling. Brackets and roman numbers mark time intervals over which net N fluxes were calculated

concentrations averaged 33.3 mmol-N kg^{-1} (Table 1), implying that NH_4^+ added in the T-NH_4NO_3 and T-NH_4 treatments (2.9 mmol-N) could increase the NH_4^+ concentration with a potential 9%. Even though amounts of NO_3^- added (1.1 mmol-N kg^{-1}, T-NH_4NO_3 and T-NO_3) were about one-third of NH_4^+ added, due to the low initial KCl-extractable NO_3^- concentrations (0.45 mmol-N kg^{-1}; Table 1), NO_3^- additions potentially more than tripled NO_3^- availability. Total inorganic-N additions measured 12, 8 and 3% of the initial inorganic-N for T-NH_4NO_3, T-NH_4 and T-NO_3, respectively.

Upon wetting and N additions, a fast response of respiration and N fluxes was observed. Average CO_2 respiration rate almost doubled from 139 mmol-C kg^{-1} day^{-1} 1 day prior to the start of the experiment ($w=115\%$) to 254 mmol-C kg^{-1} day^{-1} 3 h after rewetting the litter to $w=275\%$ ($t=0.13$; Fig. 2). No differences in respiration rates were observed between the different N treatments. For T-NH_4NO_3 and T-NH_4, the elevated respiration was accompanied by a large net N immobilization (retention, Fig. 3) of

23.0 and 8.2 mmol-N kg^{-1} day^{-1}, respectively. Recorded net N immobilization rates for T-noN and T-NO_3 were 4.9 and 1.2 mmol-N kg^{-1} day^{-1}, respectively, but these rates did not significantly deviate from 0 ($p=0.05$). Net nitrification rates differed considerably between treatments that had received NO_3^- and those that had not. Whereas T-noN and T-NH_4 showed a small net nitrification, T-NH_4NO_3 and T-NO_3 showed a relatively large net NO_3^- retention. Recorded net NO_3^- retention rates amounted to an average of 1.6 mmol-N $kg^{-1}day^{-1}$ for both treatments, implying that within 3 h after the additions about 18% of the added NO_3^- was lost. Note that NO_3^- lost as N_2O did not contribute to this net NO_3^- retention as N_2O production was taken into account in the calculation of the net nitrification rates.

Continuously moist incubations two and more days after rewetting and N addition

Even though over the course of the experiment moisture contents did not change for the MO

Fig. 2 CO_2 respiration rate for **a** moist (MO) and **b** rewetted-and-dried (WD) incubations. Brackets denote standard deviation. Horizontal brackets and roman numbers mark time intervals over which net N fluxes were calculated
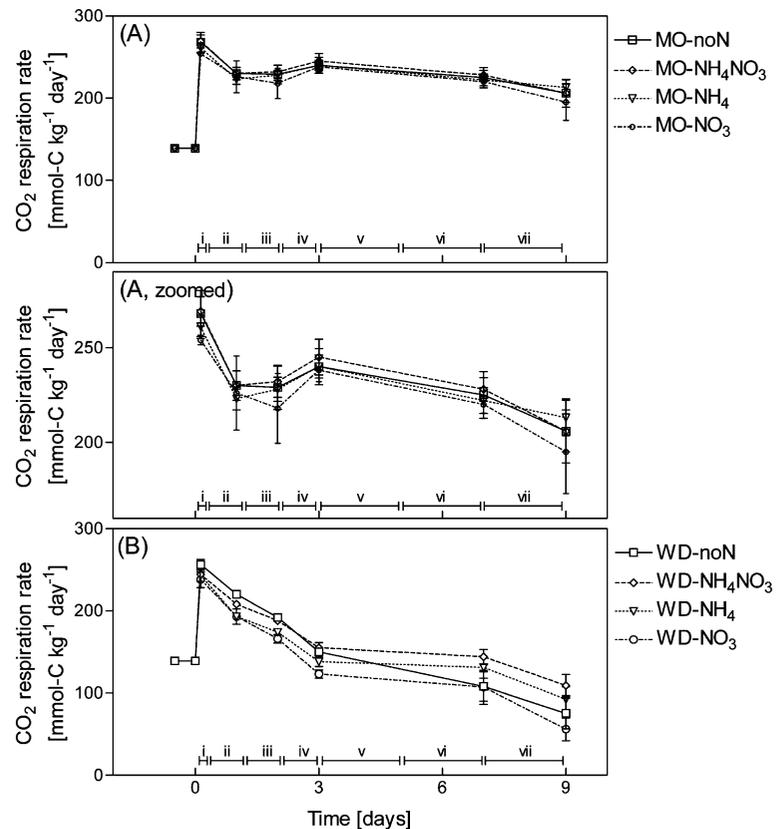
Fig. 3 Net N mineralisation **a** and net nitrification **b** rates of continuously moist incubations (*MO*). Roman numbers refer to time intervals as in Fig. 1 and Table 3. Net rates that do not significantly deviate from 0 are marked with "o" (*p*=0.05)
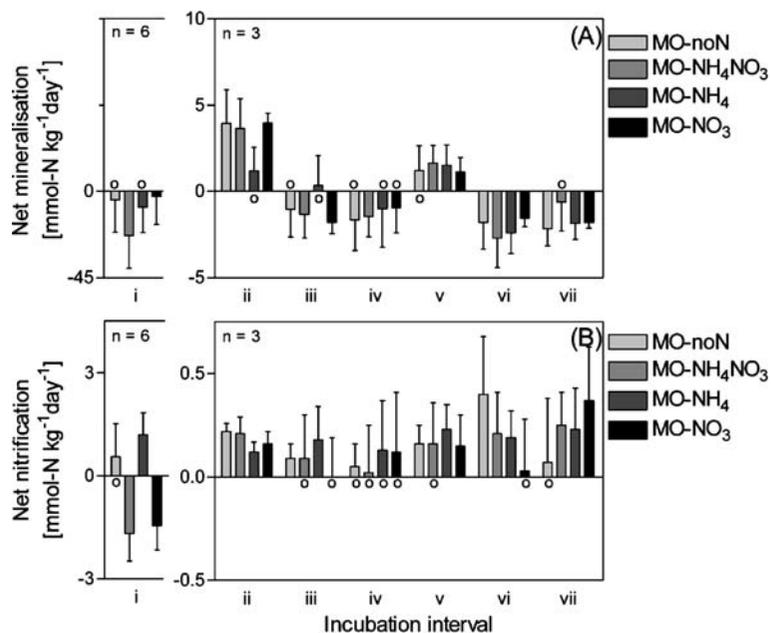
incubations, CO_2 respiration rate decreased from an average of 254 mmol-C kg^{-1} day^{-1} at $t=0.13$ to 227 mmol-C kg^{-1} day^{-1} at $t=1.0$ (Fig. 2a). No clear difference in respiration rates was observed between the different treatments. Parallel to this decrease in respiration, all MO incubations showed a net N mineralization between $t=0.13$ and 1.0 (time interval *ii*). Net mineralization rates were between 3.6 and 4.0 mmol-N kg^{-1} day^{-1} for MO-noN, MO-NH_4NO_3 and MO-NO_3, and were 1.2 mmol-N kg^{-1} day^{-1} for MO-NH_4 (Fig. 3a). Note that this latter rate did not significantly deviate from 0 ($p=0.05$).

Between $t=1.0$ and 3.0 (*ii*), the average CO_2 respiration rate somewhat increased again to 241 mmol-C kg^{-1} day^{-1}, after which it decreased to 224 and 205 mmol-C kg^{-1} day^{-1} at $t=7.0$ and 9.0 (Fig. 2a). From $t=1.0$ to 9.0, all treatments showed a net N immobilization, except between $t=3.0$ and 5.0 (*v*) and a small, but insignificant, net N mineralization for MO-NH_4 between $t=1.0$ and 2.0 (*iii*). When respiration rates at $t=5.0$, which were not determined, were in between rates measured at $t=3.0$ and $t=7.0$, *i.e.* when respiration rates decreased steadily after $t=3.0$, the temporal net N mineralization recorded between $t=3.0$ and 5.0 (*v*) corresponded with a decrease in CO_2 respiration following a temporal respiration increase. A similar relation between N mineralization and CO_2 respiration was already

observed between $t=0.13$ and 1.0 (*ii*). Effectively, over the total 9 days of incubation, all rewetted-and-moist incubations showed a net N immobilization (Table 3), which was highest for MO-noN and MO-NH_4 (both around 6 mmol-N kg^{-1}), intermediate for MO-NH_4NO_3 (5.2 mmol-N kg^{-1}) and lowest for MO-NO_3 (4.1 mmol-N kg^{-1}), although differences between treatments were not significant ($p=0.05$).

From $t=0.13$ until the end of the experiment all treatments showed a net production of NO_3^- (Fig. 3b). From day-to-day differences in net nitrification rates were observed between the treatments, but over the total period of $t=0.13$ to 9.0 no significant differences in cumulative net nitrification were detected. Cumulative net nitrification in that period ranged from 1.4 to 1.7 mmol-N kg^{-1}. At $t=0.13$, N_2O production measured about 0.08 mmol-N kg^{-1} day^{-1}, after which production rates steadily increased to between 0.17 and 0.25 mmol-N kg^{-1} day^{-1} at $t=9.0$ (Fig. 4a). This increase was faster for the NO_3^- receiving treatments (MO-NH_4NO_3 and MO-NO_3) with significantly higher N_2O production rates (0.19 mmol-N kg^{-1} day^{-1}) at $t=3.0$ than MO-noN and MO-NH_4 (0.15 mmol-N kg^{-1} day^{-1}). N_2O production rates remained significantly higher at $t=7.0$ and 9.0 for MO-NH_4NO_3, but for MO-NO_3 production rates were in the same order as those observed for MO-noN and MO-NH_4. Cumulative production of N_2O, determined over the total

Table 3 Inorganic-N ($NH_4^+ + NO_3^-$) and NO_3^- concentrations at $t=9.0$ days, and net N mineralisation, net nitrification, N_2O production and ratio of N_2O production-to-net nitrification between $t=0.0$ and 9.0 days. Concentrations in mmol-N kg^{-1}. Fluxes in mmol-N kg^{-1} (9 days)$^{-1}$

Treatment	$NH_4^+ + NO_3^-$	NO_3^-	net N mineralisation	net nitrification	N_2O production	molar ratio N_2O:net nitrification
MO-noN	26.4 (1.3)	0.71 (0.24)	-6.0 (2.1)	1.60 (0.30)	1.34 (0.16)	0.84
MO-NH4NO3	30.9 (1.8)	1.13 (0.26)	-5.2 (2.4)	1.28 (0.30)	1.69 (0.14)	1.32
MO-NH4	29.1 (0.0)	0.88 (0.35)	-6.2 (1.7)	1.86 (0.35)	1.43 (0.01)	0.77
MO-NO3	29.3 (0.6)	1.17 (0.23)	-4.1 (1.8)	1.14 (0.25)	1.51 (0.07)	1.32
WD-noN	30.4 (2.1)	1.14 (0.35)	-3.0 (2.7)	1.14 (0.37)	0.45 (0.09)	0.39
WD-NH4NO3	35.0 (2.7)	1.65 (0.16)	-2.2 (3.2)	0.69 (0.19)	0.58 (0.07)	0.84
WD-NH4	34.9 (1.0)	1.26 (0.22)	-1.3 (2.0)	1.36 (0.26)	0.54 (0.12)	0.40
WD-NO3	33.2 (8.3)	1.84 (0.92)	-1.2 (8.5)	0.75 (0.92)	0.45 (0.03)	0.60

period of incubation, increased following MO-noN $\approx$ MO-NH$_4$ < MO-NO$_3$ < MO-NH$_4$NO$_3$ (Table 3).

For MO-noN and MO-NH$_4$, about 80% of the NO$_3^-$ net produced by nitrification was lost again from the litter as N$_2$O (Table 3). For MO-NH$_4$NO$_3$ and MO-NO$_3$, N$_2$O production even exceeded net nitrification. In terms of net NO$_3^-$ loss or gain, exclusive of the additions, over the course of the experiment MO-noN and MO-NH$_4$ gained about 0.3 to 0.4 mmol-N kg^{-1} of NO$_3^-$, while MO-NH$_4$NO$_3$ and MO-NO$_3$ lost about 0.4 mmol-N kg^{-1}. Thus, whereas at $t=0.13$ NO$_3^-$ concentrations were almost three-fold higher for NO$_3^-$ receiving treatments, this differences

had largely disappeared at the end of the experiment (Table 3).

Rewetted-and-dried incubations two and more days after wetting and N addition

After the steep rise directly after the additions, CO$_2$ respiration rates (Fig. 2b) steadily decreased with decreasing moisture content. A clear correlation between respiration rate and moisture content was observed, with r^2 ranging between 0.94 and 0.99 for linear regression models of moisture vs. respiration. Cumulative CO$_2$ respiration, determined over the

Fig. 4 N$_2$O production rates for **a** continuously moist (*MO*) and **b** rewetted-and-dried (*WD*) incubations. Brackets denote standard deviation. Horizontal brackets and roman numbers mark time intervals over which net N fluxes were calculated
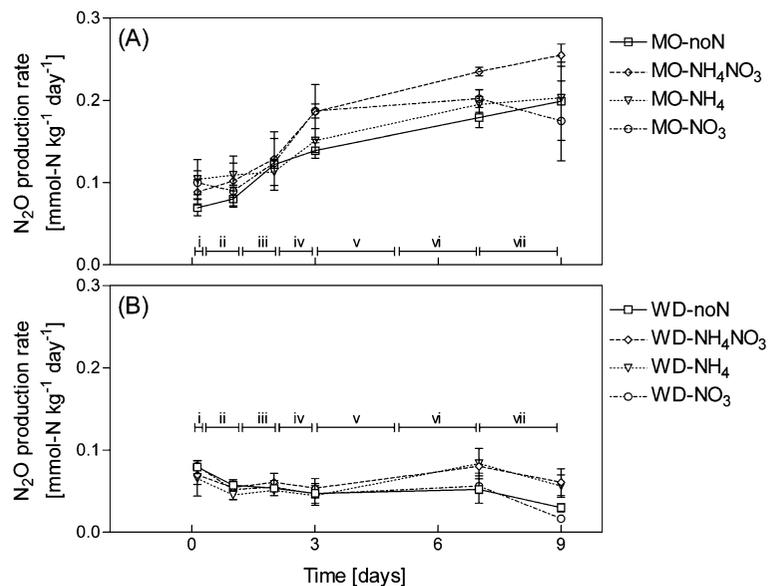
total period of incubation, was much smaller for the WD treatments (1,300 mmol-C kg^{-1}) than for the MO treatments (2,050 mmol-C kg^{-1}). At and before $t=3.0$, respiration rates were highest for WD-noN and WD-NH$_4$NO$_3$. After $t=3.0$, rates declined more strongly for WD-noN, resulting in a sequence in respiration rate at $t=9.0$ following WD-NO$_3$ ≈ WD-noN ≤ WD-NH$_4$ ≈ WD-NH$_4$NO$_3$. Cumulative CO$_2$ respiration estimated over the total period of incubation increased following WD-NO$_3$ < WD-noN ≈ WD-NH$_4$ < WD-NH$_4$NO$_3$.

Similar to the MO incubations, N was net mineralized between $t=0.13$ and 1.0 (time interval *ii*, Fig. 5a). Subsequently, between $t=1.0$ and 9.0, no clear patterns in net N mineralization could be detected, *i.e.* temporary and small net N mineralizations were alternated by small net N immobilizations. Effectively, over the 9 days of incubation, all treatments showed a small net N immobilization (between 1.2 and 3.0 mmol-N kg^{-1}, Table 3), but this immobilization was significant only for WD-noN. At $t=9.0$, inorganic-N concentrations were at the same level as before the start of the experiment, that is between 30 and 35 mmol-N kg^{-1}.

From $t=0.13$ until the end of the experiment all treatments showed a net nitrification (Fig. 5b). Again, from day-to-day differences in net nitrification rates were observed between the different treatments, but

no clear trend was noticed. In addition, many of the recorded nitrification rates were insignificant. Total net nitrification, measured cumulative between $t=0.13$ and 9.0, amounted between 0.8 and 1.2 mmol-N kg^{-1}, with no significant difference between the treatments. This net nitrification was lower than for the MO treatments where, for the same period, net nitrification was between 1.4 and 1.7 mmol-N kg^{-1}.

Unlike the MO incubations, N$_2$O production rates remained at a constant low level of 0.02 to 0.08 mmol-N kg^{-1} day^{-1} (Fig. 4b). Between $t=0.13$ and 3.0, production rates were the same for all treatments, but from $t=7.0$ to 9.0 rates for WD-noN and WD-NO$_3$ were significantly lower than for the other two treatments. This lower N$_2$O production rate did not correspond with lower NO$_3^-$ concentrations, but did coincide with a lower CO$_2$ production rate for these two treatments. Over the total period of incubation, for all treatments net nitrification was larger than the cumulative N$_2$O production (Table 3). Thus, all treatments showed a net increase in NO$_3^-$ concentration and despite lower total net nitrification this increase was larger than for the MO series. At $t=9.0$, average NO$_3^-$ concentrations for the NO$_3$ receiving treatments were still 0.5 mmol-N kg^{-1} higher than for the non-receiving treatments (Table 3), but due to high standard deviations for WD-NO$_3$, only differences between WD-NH$_4$NO$_3$ and WD-

Fig. 5 Net N mineralisation **a** and net nitrification **b** rates of rewetted-and-dried incubations (*WD*). Roman numbers refer to time intervals as in Fig. 1 and Table 3. Net rates that do <u>not</u> significantly deviate from 0 are marked with "o" ($p=0.05$)
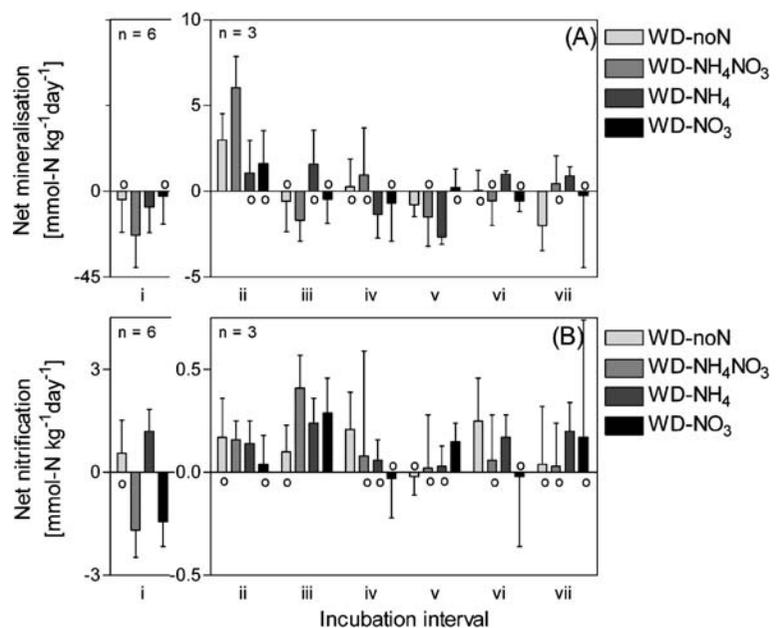
noN, and between WD-NH$_4$NO$_3$ and WD-NH$_4$ were significant.

Discussion

This discussion section is structured around four main topics. These are the observed rapid N retention directly after N additions, the linkage between CO$_2$ respiration and net N mineralization, the potential of the fresh litter for N$_2$O production, and, finally, the implications of N additions for N retention and loss at the Speuld forest.

Rapid N retention directly after N additions

A relatively large net N immobilization was observed in the first 3 h after the additions (time interval i) when NH$_4^+$ had been added to the fresh litter. Similar, NO$_3^-$ receiving samples showed a net retention of NO$_3^-$, summing up to about 18% of the NO$_3^-$ added.

Rapid immobilization of added NO$_3^-$ has been detected in other studies, even for forests that are subject to chronic N deposition (*e.g.*, Berntson and Aber 2000; Dail et al. 2001; Fitzhugh et al. 2003). For mixed hardwood and pine stands in Northern America, Berntson and Aber (2000) reported as much as 34–62% of the added ^{15}NO$_3^-$ to be immobilized by the forest floor within 15–45 min after the additions. For a mixed hardwood stand of the same forest, Dail et al. (2001) found that between 30 and 60% of ^{15}NO$_3^-$ added to forest floor material disappeared from the inorganic-N pool within 15 min after the additions. Of this immobilized ^{15}NO$_3^-$, about 95% was recovered as DON and it was argued that an abiotic process was responsible for this N transformation (Dail et al. 2001). At present, there is much discussion about the true occurrence of abiotic NO$_3$ incorporation in soils and reference is made to the debate between Colman and Davidson (Colman et al. 2007, 2008; Davidson et al. 2008).

It remains unclear what caused the rapid retention of the added N in our study, which was not designed to detect the exact pathways of the added N. The results do, however, show that rapid retention has place in fresh litter, and that this could be an important pathway for NO$_3^-$ retention in this N saturated Douglas fir forest.

Respiration and net N mineralization responses to wetting

The wetting of the litter at the start of the experiment resulted in a steep rise in CO$_2$ respiration, indicating an increase in microbial activity and a probable higher microbial demand for N upon wetting. In the days following the additions, CO$_2$ respiration and N mineralization seemed closely linked, with N being net mineralized when CO$_2$ respiration rates (temporarily) decrease.

Almost immediate increase in respiration after rewetting has been recorded by others (*e.g.*, Borken et al. 2003), though recovery of CO$_2$ respiration to optimal levels may also take a few days (Muhr et al. 2008). Secondary peaks or oscillation periods of CO$_2$ respiration, as observed for the MO incubations, have also been reported by Clein and Schimel (1994) for birch litter that was kept moist after an initial drying. They, similar to Lund and Goksøyr (1980), attributed this to species diversity within the microbial community, with some species reacting to wetting slower than others. The recorded temporal elevated CO$_2$ respiration (period i) is well-known from the literature and is often referred to as the Birch-effect, after the pioneering work of Birch (1964). Several different processes may be responsible for this effect, as summarized by Muhr et al. (2008, p.726).

Our results contrast with the findings of Pulleman and Tietema (1999), who performed drying and wetting experiments with older (F2 horizon) litter from the same forest stand. They also observed a rapid increase in respiration rates upon rewetting, but in their experiment this coincided with a large, temporary net N mineralization flush rather than a net N immobilization. This different response to rewetting can be explained largely by the difference in magnitude of the drying prior to wetting in both experiments and differences in substrate quality and microbial community structures between both types of litter. The extreme drying treatment ($w = 10\%$ prior to rewetting) in the experiment of Pulleman and Tietema (1999) generated easily decomposable (biomass and non-biomass derived) substrate, which before drying was not generally present in the litter. After wetting, gross mineralization increased rapidly, but increase in growth of the microbial community and subsequent gross immobilization was delayed as the microbial community had to adapt to the new,

easily decomposable substrate. Thus, a net N mineralization flush was observed directly after the rewetting. In our experiment, in which actual field moisture conditions of the Speuld forest floor were more closely mimicked, drying prior to rewetting was not that severe ($w=115\%$) and probably did not have much effect on the substrate quality. In addition, our fresh litter already contained substantial amounts of easily decomposable organic matter, as shown by the relatively high respiration rates in our experiments. As such, after wetting, the microbial community did not have to adapt to the substrate and there was no delay in microbial growth after wetting.

Between $t=0.13$ and 1.0, respiration rates somewhat decreased for MO and WD treatments. Likewise, for the MO incubations between $t=3.0$ and 5.0, respiration rates decreased after an increase in the days before. We hypothesize that the net N mineralization observed in these periods was due to ongoing activity of extracellular enzymes that were produced in surplus when the microbial community was still growing. Exoenzymes are produced by microorganisms to catalyze the breakdown of organic matter (Michel and Matzner 2003; Schimel and Weintraub 2003). Hence, there was a time lag between decrease in microbial demand (gross immobilization) and decrease in enzyme activity (gross mineralization), resulting in a temporary net N mineralization. During the drying phase of the WD treatments, respiration rates steadily decreased. Small and mostly insignificant net N immobilization was alternated with insignificant net N mineralization, indicating a tight balance between N immobilization and mineralization during this period of decreasing microbial activity.

N$_2$O production potential in fresh litter

The relatively high, and at the end of the experiment still increasing N$_2$O production in the MO incubations indicate that fresh litter from this forest has a high potential for N$_2$O production. However, as shown by lower N$_2$O production in the WD series, that more closely mimicked the actual moisture conditions in the Speuld litter, this potential is probably not fully exploited in the field. Still, even for the WD treatments, N$_2$O production was relatively high compared to net nitrification, with approximately half of the net

produced NO_3^- being lost again as N_2O. In these calculations, it was assumed that none of the N_2O produced was transformed into N_2 (N_2:N_2O production ratio of 0:1). We realize that this assumption may be too strict, even though relative to N_2O, production of N_2 is low under acidic conditions (Blackmer and Bremner 1978; Wolf and Brumme 2003). Using a higher N_2-to-N_2O production ratio in our calculations of gaseous loss of NO_3^-, however, would have only increased the amounts of NO_3^- lost from the system as N_2 and N_2O.

The fairly constant N_2O production rates throughout the WD incubations are in accordance with the theory of Van der Lee et al. (1999), who argued that denitrification in anoxic microsites within decomposed Douglas fir needles may be responsible for N_2O production in well-drained forests. They observed that this anoxic organic matter fraction of the forest floor did not significantly decrease with decreasing water content, which may explain the continuing N_2O production while our litter dried out. Martikainen and De Boer (1993), on the other hand, showed that aerobic production of N_2O by nitrification may as well occur in litter of our forest type, and we cannot resolve whether this mechanism or denitrification in microsites was responsible for the observed N_2O production.

Similar, for the MO treatments it is not clear which of the two mechanisms accounted for the N_2O production. However, we cannot think of sound reasons why N_2O production rates would increase with the duration of the experiment when nitrification was the prime mechanism. Martikainen and De Boer (1993) indeed measured a constant N_2O production rate in their litter, which was incubated under similar moisture conditions ($w=280\%$) as our litter, although it should be mentioned that they used F instead of L horizon material. When denitrification was responsible for N_2O production in our experiment, N_2O production conditions were in principle favorable, because of the presence of easily decomposable organic matter. Concurrent to Van der Lee (2000), we hypothesize that due to high O_2 consumption, as shown by the continuously high CO_2 respiration rates, over the course of the experiment anoxic conditions may have developed in water filled pores outside of litter particles. The continuous increase in N_2O production can then be explained by a continuous increase in anoxic volume.

Implications of N additions for N retention and loss at the Speuld forest

Even though our experiment comprised of 9 days only, it gives some information on possible N sources and sinks in fresh Douglas for litter on the longer term. Here, focus is on the WD experiments as these more closely mimic actual moisture conditions in the Speuld litter.

In the first few hours after additions, an apparent relation between N mineralization and amount of N added was found, *i.e.* a net N immobilization was observed when NH_4^+ had been added to the litter. After 9 days of incubation, however, any effect of this short-term reaction of N mineralization to N addition had disappeared. Observed net N immobilizations, determined over the total incubation period, were relatively small and any differences between treatments were insignificant. It thus seems unlikely that immobilization is an important N sink in the Speuld LF1 horizon.

Even though NO_3^- was net produced over the period of incubation, two pathways of inorganic-N loss were associated with NO_3^-. Firstly, following Dail et al. (2001), when the rapid retention of added NO_3^- directly after additions was due abiotic transformation of NO_3^- into DON, this could be a longer-term sink for NO_3^-. Secondly, even in the WD incubations, about half of the NO_3^- net produced was transformed into N_2O, denoting the relative importance of denitrification in exporting NO_3^- from the LF1 horizon. The contribution of both sinks to the total N balance of the Speuld forest, however, remains relatively small as dry mass of the LF horizon is only 12% of the total dry mass of the forest floor (Wessel and Tietema 1995). Rough estimates demonstrate that the maximum loss of NO_3^- via incorporation into DON or loss as N_2O is about 1 kg-N ha^{-1} yr^{-1} for each NO_3^- sink, respectively, which is small compared to the 31 kg-N ha^{-1} yr^{-1} of NO_3^- that are leached from the soil at 90 cm depth.

Conclusions

Upon moisture, NH_4^+ and NO_3^- additions to fresh Douglas fir litter, a series of transformations occur of which most notable are a rapid retention of added NH_4^+ and NO_3^- and a sudden increase in CO_2 respiration. In days following wetting, inorganic-N mineralization and CO_2 respiration seem closely connected, and our results suggest that net N mineralization occurs only when respiration rates decrease after an initial respiration increase. Fresh Douglas fir litter has a high potential for production of N_2O, but this potential is exploited only under prolonged wet conditions, which do not often occur in the field. Still, dried-and-rewetted litter acts partly as a sink for NO_3^-, through rapid incorporation of added NO_3^- in the soil and gaseous loss of NO_3^- as N_2O.

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