Nitrogen release in decomposition of boreal mor and peat as affected by enchytraeid worms

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Mor, slightly decomposed peat and highly decomposed peat, two soil types of each, were incubated for 154 days in the laboratory at +15 °C with and without enchytraeid worms, which functionally are the most influential faunal group in boreal forests. We quantified the release rates of organic and inorganic nitrogen (N) in dissolved and extractable forms from decomposing organic matter and explored the effects of enchytraeid worms on the release rates. About 80% of the dissolved N was released in the form of NH_4^+-N, except in mor without worms and in slightly decomposed peat with and without worms, where the net release from soil solution was in the form of dissolved organic N (DON). The majority of DON was in the high molecular weight fraction. In the presence of worms, the mineralization rate of N was highest in mor and in the absence of worms in highly decomposed peat. The large initial DON pool may explain the high mineralization rate in highly decomposed peat. The changes in the soil-solution N pool were small compared to the changes in extractable N pool which emphasizes the importance of the adsorbed N in soil N dynamics. Because N release through decomposition is the major component of N balance in forested catchments, the results of the study can be used for improving models of catchment scale N dynamics.

Introduction

Most of N in boreal forest soil is bound to dead organic matter, and to a smaller extent to

living roots, microbes and fauna (Schulten and Schnitzer 1998). As a result of decomposition by soil microbes and fauna, a small proportion of the total N is dissolved in soil solution, mainly

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as dissolved organic N (DON) and ammonium (NH_4^+-N). Soil microbes are known as the primary decomposers of organic matter while soil fauna, such as protozoans, nematodes, a variety of soil microarthropods and enchytraeid worms, enhance the release of N by fragmenting organic matter into smaller particles and by grazing upon microbes (Setälä and Huhta 1991, Bardgett and Chan 1999). Functionally the most influential faunal group in boreal upland and peatland forest soils is enchytraeids (Laakso and Setälä 1999, Silvan *et al.* 2000), of which more than 95% is comprised of one species, *Cognettia sphagnetorum* (Abrahamsen 1972, Nurminen 1967).

In many boreal forested catchments, the major N input to surface waters occurs in the form of DON (Ahtiainen and Huttunen 1999, Perakis and Hedin 2002). One factor affecting the export of DON from soil is the size of DON molecules. The export of labile low molecular weight DON (LMW-DON) is likely to be small because the half-life time of labile fraction is in the order of few days (Kalbitz *et al.* 2003a), while a residence time of soil water in forested catchments can be several months (Lepistö 1994). High molecular weight DON (HMW-DON) consists mainly of refractory compounds with low biodegradability. The refractory compounds originate from highly decomposed organic matter and microbial metabolites (Kalbitz *et al.* 2003b), while fresh litter is the main source of labile fraction (Kalbitz *et al.* 2003a, Kiikkilä *et al.* 2006). Although soil fauna are known to enhance biodegradation from less labile pools (Fox *et al.* 2006, Briones *et al.* 2007), little is known about their relative importance as affecting the release of refractory and labile DON fractions in organic soil.

The quantification of organic matter decomposition is a prerequisite for understanding the mechanisms of DON export to water courses and for developing realistic process-based solute-transport models. Laurén *et al.* (2012) conducted a laboratory experiment to support development of the decomposition model ROMUL (Chertov *et al.* 2001) with a special emphasis on facilitating the use of the decomposition model in solute-transport applications. Laurén *et al.* (2012) studied the release rates of NH_4^+-N, LMW-DON and HMW-DON from the boreal-forest floor and interpreted the results from the water quality

point of view. However, in the study by Lauren *et al.* (2012), the release rates from only one type of mor humus were explored, whereas a larger, catchment scale approach calls for knowledge about the N release rates from other mor and peat soil types as well. In the current study, we aimed at filling this gap in knowledge by using the same methodology as in Laurén *et al.* (2012). We conducted a controlled incubation experiment to study the N release from decomposing mor, slightly decomposed peat, and highly decomposed peat, two soil types of each. During the incubation, we measured the release of N in organic and inorganic forms and divided DON into the LMW and HMW fractions. We hypothesized (1) that the differences between the decomposing materials are reflected in the release of NH_4^+-N and DON, and (2) that the presence of soil fauna, represented by enchytraeids, enhance the overall release of N. Because *Sphagnum* residues typically decompose slowly (Johnson and Damman 1993), we expected that release rates of N are higher in mor than in peat. Furthermore, we expected that slightly decomposed peat releases more N than highly decomposed peat. Finally, we predicted that, due to the more preferential food resources and moisture conditions (Didden 1993, Silvan *et al.* 2000), enchytraeids are more influential in mor than in peat, and more influential in slightly decomposed peat than in highly decomposed peat.

Material and methods

Study sites and sampling

Soil sampling for the laboratory experiment was conducted in June 2008 in Sotkamo, eastern Finland. Long-term (1971–2000) mean annual precipitation in the area was 585 mm with about 40% falling as snow, and mean annual air temperature was +1.7 °C (Drebs *et al.* 2002). In the experiment, we used the most common organic soil types in Finland. The typical upland forest types are Mesic heath forest and Sub-xeric heath forest, which together contribute to more than 70% of the upland mineral soil areas (Tomppo 2000). The organic soil horizon at these sites is mor humus. The most common peat types are

Sphagnum- and *Carex*-dominated peats representing, respectively, 49% and 37% of peatlands in Finland (Virtanen *et al.* 2003). Altogether six organic soil types were collected: medium and low fertility type mor, slightly and highly decomposed *Carex–Sphagnum* peat and slightly and highly decomposed *Sphagnum* peat. Mor samples were collected from the adjacent catchments of Kangasvaara and Kangaslampi (63°51´N/28°58´E) (Finér *et al.* 1997). The ground vegetation cover was classified as the *Vaccinium–Myrtillus* type in Kangasvaara and as the *Empetrum–Vaccinium* type in Kangaslampi (Cajander 1949). In Kangasvaara, the thickness of the organic horizon ranged from 5 to 9 cm and in Kangaslampi from 3 to 5 cm. The forest at both sites was old-growth Norway spruce (*Picea abies*) mixed mainly with Scots pine (*Pinus sylvestris*). The mean height and the mean stem volume of the stands were 23 m and 270 m^3 ha^{-1}, respectively, at the Kangasvaara site, and 14 m and 130 m^3 ha^{-1}, respectively, at the Kangaslampi site. The samples of *Carex–Sphagnum* and *Sphagnum* peat were collected from drained pine bogs in the Koivupuro and Suopuro catchments (63°52´N/28°39´E) (Ahtiainen and Huttunen 1999), where the dominant tree species was Scots pine and the ground vegetation cover represented the dwarf-shrub type (Cajander 1949). The mean height and the mean stem volume of the stands were 14 m and 65 m^3 ha^{-1}, respectively, in Koivupuro, and 6 m and 27 m^3 ha^{-1}, respectively, in Suopuro. In Koivupuro, the peat layer depth was 1–5 m and in Suopuro 1.5–2 m. The peat samples were collected from the surface layer (to ca. 20 cm depth) that represented slightly decomposed peat (H3–H4 on the von Post (1922) scale of decomposition) and from the underlying layer (in ca. 20–40 cm depth) that represented highly decomposed peat (H6–H7 on the von Post scale of decomposition). At each site, a topographically even location with homogeneous vegetation between the trees was selected for the sampling spot.

A total of 48 soil samples, eight for each soil type, were used for the incubation. A cylindrical piece (diameter 20 cm, height 9–15 cm) was cut from the organic layer with a knife to fit the piece in a plastic container (diameter 20 cm, height 20 cm). All the pieces were cut close to each other. Living, aboveground vegetation was carefully removed from all samples. Due to thin mor layer at the low-fertile upland site, the samples of this material were constructed of several layers placed in the container layer by layer until the thickness of the sample was ca. 10 cm. This was conducted to obtain a sufficient soil volume for soil solution sampling during the incubation. Parallel soil cores, four for each soil type, were collected for analyzing basic characteristics and extractable N contents of the soils. Prior to analyzes and incubation, all samples were stored in –20 °C (*see* the defaunation process below). In addition, mor material was collected for the extraction of enchytraeid worms, which were later introduced into half of the soil containers as described later. Prior to the worm extraction, the soil was stored in +4 °C. The main procedures of the experiment are shown in Fig. 1.

Incubation environment and soil analyses before the incubation

Before starting the experiment, meso- and macrofauna in the soil containers were killed by a defaunation process, in which successive freezing (down to –20 °C) and thawing of the containers was repeated twice. The defaunation treatment is likely to cause a momentary N flush from the soil, which was taken into account in the statistical data analysis (described later). After defaunation, the soil water content in each container was adjusted to correspond to the field capacity by wetting the containers with deionized water until water started to drop through the hole in the bottom of each container. Surplus water was let to drain until the dropping ceased. Thereafter, the holes were closed and the containers weighted. The containers were placed in a dark growth chamber (GR77, Conviron Controlled Environments Ltd., Canada) having a constant ambient temperature of +15 °C and a relative humidity of 80%. The incubation temperature was the approximated mean summer (June–August) air temperature in the study region (Drebs *et al.* 2002). Half of the containers, four of each soil type, were inoculated with enchytraeid worms. Enchytraeids had been extracted from the additional mor material using
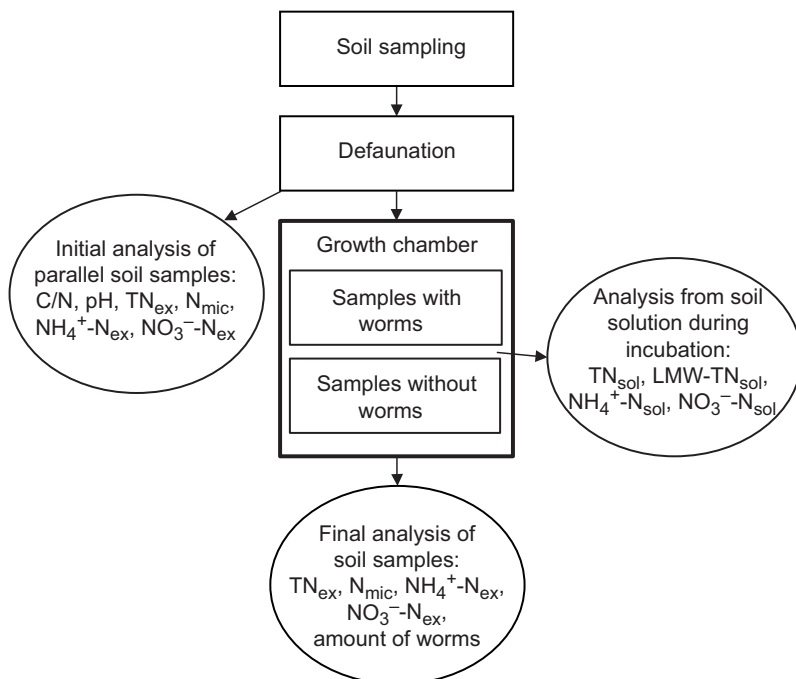
Fig. 1. The main procedures of the experiment.

the wet funnel method (O'Connor 1962). About 50 worms were introduced into half of the containers at the beginning of the incubation, and further 50 worms monthly to assure the presence of the enchytraeid population in the containers. In total ca. 8000 indiv. m^{-2} or 0.1 g m^{-2} expressed as dry mass of worms were inoculated. The total number of worms in the soil was determined at the end of the incubation.

The soil C/N ratio, pH, and contents of extractable N compounds were determined at the beginning of the experiment using parallel soil samples. Total C and N contents in the soils were determined using a CHN analyzer (CHN-2000, LECO Corporation, USA) and soil pH was measured in a suspension of soil in H_2O (1:2, v:v) (Table 1). Extractable total N, NH_4^+-N and $(NO_2^- + NO_3^-)$-N were extracted with 1 M KCl (Table 2). Hereafter, $(NO_2^- + NO_3^-)$-N is referred to as NO_3^--N, and the compound contents measured from the extract are indicated by the subscript "ex". N compounds were analyzed with

Table 1. Basic characteristics of the studied organic soils given as means ± SDs ($n = 8$, except for C/N and pH; $n = 4$). The values followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$).

Soil type	C/N	pH	Water content* (vol-%)	Bulk density* (kg m^{-3})	LOI* (%)
Mor					
(1) medium fertility	39.2 ± 0.6[a]	3.82 ± 0.04[b]	33.1 ± 2.9[a]	87.2 ± 17.9[bc]	95.3 ± 0.5[b]
(2) low fertility	43.9 ± 2.9[a]	3.76 ± 0.04[a]	39.2 ± 4.8[ab]	94.8 ± 6.9[c]	93.6 ± 1.9[a]
***Carex–Sphagnum* peat**					
(3) slightly decomposed	37.7 ± 0.6[a]	3.72 ± 0.02[a]	47.2 ± 7.2[b]	59.8 ± 4.4[a]	97.6 ± 0.3[c]
(4) highly decomposed	40.0 ± 0.6[a]	3.83 ± 0.01[b]	73.9 ± 4.4[c]	145 ± 13.2[e]	98.5 ± 0.2[c]
***Sphagnum* peat**					
(5) slightly decomposed	41.6 ± 2.1[a]	3.84 ± 0.01[b]	75.8 ± 5.4[c]	75.7 ± 5.5[b]	98.0 ± 0.7[c]
(6) highly decomposed	38.0 ± 1.0[a]	3.80 ± 0[b]	76.2 ± 8.3[c]	127 ± 5.2[d]	98.6 ± 0.3[c]

* measured at the end of the incubation.

a spectrophotometer (FIA-Star 5000 Analyzer, FOSS Tecator, Denmark). Extractable organic N (ON_{ex}) content was calculated by subtracting NH_4^+-N_{ex} and NO_3^--N_{ex} from the total extractable N (TN_{ex}) content. Microbial biomass N (N_{mic}) was determined using the fumigation-extraction method (Brookes *et al*. 1985), as described in Laurén *et al*. (2012).

Soil solution sampling and analyses during the incubation

During the incubation of 154 days, soil solution samples for analyses of dissolved N compounds were regularly collected from the containers using suction samplers (MacroRhizon with syringe, Eijkelkamp, The Netherlands). The dissolved compound contents other than DON are later in the text indicated by the subscript "sol". The suction sampler consisted of a polymeric porous tip (length 9 cm and diameter 4.5 mm), attached to a removable syringe generating suction of approximately 100 kPa. The mean pore size of the sampler tip material was 0.1 μm, therefore the registered N content must be slightly lower than the content of solution

filtered through a widely used 0.45 μm filter. However, the small pore size prevents air from bubbling into the soil solution sampler in macroporous soils such as mor, and was therefore suitable for this study. Two sampling tips were vertically inserted into each container. In each sampling event ca. 100 ml of soil solution was collected, which took 2–3 days. The mass of the solution sample was measured before analysis. The sampling was repeated eight times at 2–6 week intervals, with the shortest intervals at the beginning of the experiment. Water lost via evaporation and the soil solution sampling was compensated once a week by adding deionized water to the containers until the original mass was reached.

Soil solution samples were divided into two parts. One part was filtered (Amicon Stirred Cell model 8400, Millipore Corporation, USA, pressure 1.5–2 bar) through an ultrafiltration membrane with a nominal molecular weight limit of 1 kDa. In the filtration, two thirds of the load volume in a stirred cell was allowed to pass through the membrane. The ultrafiltered fraction represents the LMW fraction of the dissolved N. The other part remained unfiltered. Dissolved total N (TN_{sol}), NH_4^+-N_{sol} and NO_3^--N_{sol} were

Table 2. Means ± SDs of initial pools (μg g^{-1} dw) of total nitrogen (Total N), microbial biomass N (N_{mic}), total N in extract (TN_{ex}) and soil solution (TN_{sol}), low molecular weight TN in soil solution (LMW-TN_{sol}), organic N in extract (ON_{ex}) and soil solution (DON), ammonium in extract (NH_4^+-N_{ex}) and soil solution (NH_4^+-N_{sol}), nitrate in soil solution (NO_3^--N_{sol}), and the number of enchytraeids at the end of the incubation in soils.

	Mor		*Carex–Sphagnum* peat		*Sphagnum* peat	
	medium fertility	low fertility	slightly decomposed	highly decomposed	slightly decomposed	highly decomposed
Total N (*n* = 4)	13200 ± 408	11775 ± 723	14300 ± 216	14500 ± 141	14275 ± 4654	15050 ± 943
N_{mic} (*n* = 4)	465.3 ± 15.8	444.0 ± 38.8	285.6 ± 101.6	42.8 ± 32.6	559.3 ± 60.3	46.1 ± 21.7
TN_{ex} (*n* = 4)	315.3 ± 14.1	346.3 ± 60.6	415.8 ± 17.3	116.3 ± 9.3	404.3 ± 49.9	137.8 ± 32.8
TN_{sol} (*n* = 8)	10.8 ± 5.8	21.3 ± 12.5	40.2 ± 11.8	25.2 ± 6.0	50.7 ± 10.6	29.2 ± 9.1
LMW-TN_{sol} (*n* = 8)	6.4 ± 4.4	14.1 ± 9.2	27.7 ± 6.9	9.7 ± 4.0	21.0 ± 5.1	10.6 ± 3.7
ON_{ex} (*n* = 4)	286.3 ± 15.5	305.7 ± 46.5	375.6 ± 14.0	102.8 ± 9.3	373.4 ± 50.0	121.1 ± 34.3
DON (*n* = 8)	5.2 ± 1.6	5.9 ± 2.4	8.0 ± 3.4	17.7 ± 3.5	17.2 ± 9.2	19.9 ± 6.7
NH_4^+-N_{ex} (*n* = 4)	29.0 ± 3.6	40.6 ± 15.6	40.1 ± 4.3	13.5 ± 0.7	30.9 ± 0.6	16.7 ± 2.4
NH_4^+-N_{sol} (*n* = 8)	5.7 ± 5.3	15.3 ± 10.5	32.2 ± 11.0	7.5 ± 3.0	33.5 ± 5.0	9.2 ± 4.1
NO_3^--N_{sol} (*n* = 8)	0.03 ± 0.02	0.03 ± 0.02	0.07 ± 0.06	0.03 ± 0.003	0.07 ± 0.04	0.04 ± 0.02
Enchytraeids (× 10³ indiv. m^{-2}, *n* = 4)*						
depth 0–5 cm	77.7 ± 35.8	106 ± 28.7	18.6 ± 12.9	13.4 ± 7.4	19.2 ± 4.5	8.9 ± 5.7
depth 5–10 cm	37.9 ± 13.0	70.6 ± 17.9	8.4 ± 18	8.5 ± 6.0	7.4 ± 4.8	5.0 ± 4.0
depth 10–15 cm	–	–	7.9 ± 3.0	5.9 ± 4.7**	9.3 ± 5.1**	6.9 ± 4.3

* Value measured at the end of the incubation, ** *n* = 2.

determined from the unfiltered samples (FIA-Star 5000 Analyzer); LMW-TN$_{sol}$ was also analyzed from the ultrafiltered samples. The DON content was calculated as the difference between TN$_{sol}$ and inorganic N contents in the solution. Similarly, LMW-DON corresponds to the difference between LMW-TN$_{sol}$ and inorganic N in the solution. However, there was some analytical uncertainty connected to the determination of LMW-DON. We found that not all inorganic N passed through the 1 kDa filter membrane, as the NH$_4^+$-N$_{sol}$ concentration found in the unfiltered solution was occasionally higher than the filtered LMW-TN$_{sol}$ concentration, most often in peat solution samples. Probably the HMW molecules bound some inorganic N blocking their pass through the membrane. Assuming that the same amount of inorganic N is retained on the membrane in each filtration, the results can be used in the calculation of LMW release rates. Because this assumption is arguable, we use the LMW results only as a rough estimation of the size of the LMW-DON pool and the direction of the change in the LMW-DON pool. The LMW-DON pool was however small; in 73% of the solution samples, the LMW-DON concentration was below 0.1 mg l^{-1} (the detection limit of TN$_{sol}$). The concentration of NO$_3^-$-N$_{sol}$ was below the detection limit (0.01 mg l^{-1}) in 30% of the analyzed solution samples, and for those samples a value equal to half of the detection limit was used in further calculations.

Soil analyses at the end of the incubation

The fresh volume and mass of the soil in the containers were measured at the end of the experiment. The soil from each container was vertically cut into four equal sectors. The first sector was used to determine the soil volumetric water content by weighing it before and after drying in 105 °C. It was further used to determine the loss on ignition at 550 °C. The second sector was cut horizontally into 5-cm-thick slices, from which enchytraeid worms were extracted using the wet funnel method. The extraction was also conducted for the containers without the worm inoculations to verify the absence of enchytraeids. The third sector was used for the analysis of

TN$_{ex}$, NH$_4^+$-N$_{ex}$, NO$_3^-$-N$_{ex}$, and N$_{mic}$. The fourth part was stored for possible further analyses.

Data processing and statistical methods

The setup of the experiment was based on the assumption that on the time scale of the experiment duration, the net release of the measured N compounds is constant and can thus be described by a linear model. The assumption is supported by the fact that the ambient environmental conditions remained constant and the incubation time was short enough to allow only a small fraction of the total organic matter to decompose. The measured mass loss during the incubation was less than 3% of the initial dry mass (M. Lappalainen unpubl. data). At the beginning of the incubation, the NH$_4^+$-N$_{sol}$ content in mor, and DON content in peat shortly decreased (Fig. 2). The decrease could result from strong microbial assimilation after the nutrient flush caused by defaunation. Therefore, the data from the first two sampling dates were excluded from all statistical analyses. A similar initial decrease was reported by Park *et al.* (2002) and Laurén *et al.* (2012), who excluded the first sampling from their data analyses. The measured N quantities were standardized by dividing the quantity by the dry mass of the soil. The quantities of DON, NH$_4^+$-N$_{sol}$ and NO$_3^-$-N$_{sol}$ removed from the soil containers in soil solution samplings were taken into account in the calculation by adding the removed quantity to the measured pool.

The measurements form a hierarchical data structure, where the levels of the hierarchy are the sampling events, the containers, the soil types, and the worm treatments. In this setup, two consecutive sampling events from the same soil container are not independent from each other. Therefore, a mixed linear model application, which allows for analysis of multi-level hierarchical datasets (Goldstein 1995), was used in the statistical analyses. The principles for the calculation of N release rates follow the methodology presented in Laurén *et al.* (2012). The release rates of NH$_4^+$-N$_{sol}$, NO$_3^-$-N$_{sol}$, DON, and LMW-DON and their confidence intervals were calculated by using the following mixed linear model:
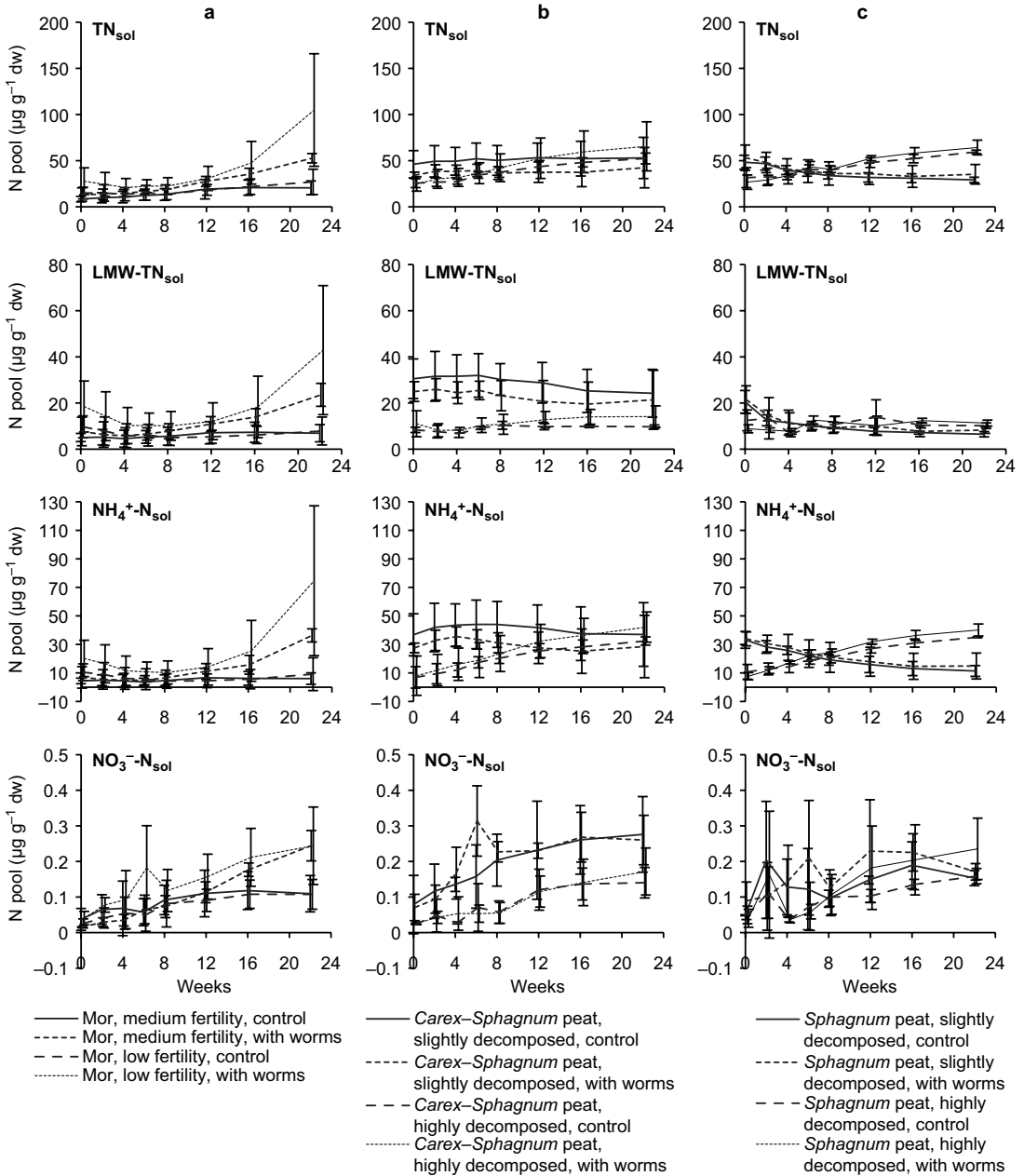
Fig. 2. Mean values and standard deviations of the measured N pools during the 22 weeks incubation experiment (n = 4). Different soil types, incubated without worms (control) and with worms, are presented for (**a**) mor types, (**b**) *Carex–Sphagnum* peat types, and (**c**) *Sphagnum* peat types.

$$Q_{ijkm} = \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_{ij}t_{ijkm} + a_{ijk} + c_{ijk}t_{ijkm} + e_{ijkm} \quad (1)$$

where Q_{ijkm} is the compound content (μg g^{-1} dw) in the soil type i, the treatment j (worms, without worms), the container k, and the sampling event m. In the fixed part of the model, α_i is the effect of the soil type i, β_j is the effect of the treatment j, $\alpha\beta_{ij}$ is the interaction between the soil type i and treatment j, γ_{ij} is the slope for the soil type i and the treatment j representing the rate of the compound release (μg g^{-1} d^{-1}), and t_{ijkm} is the time (days) from the beginning of the experiment. The

random part of the model having zero expectation value includes the intercept a_{ijk}, the slope c_{ijk}, and the residual term e_{ijkm}. Residual variance (v_{ij}) varied between the soil types and the worm treatments, and therefore the analyses were conducted with weights $1/v_{ij}$. Pairwise contrasts were used to test the differences between the mean release rates of the soil types and the worm treatments separately for each soil type.

The release rates of $NH_4^+-N_{ex}$ and ON_{ex}, and the accumulation rate of N_{mic} were analyzed with a linear model described by Eq. 2. $NO_3^--N_{ex}$ content was in all analyzed samples below the detection limit and therefore it was left out from the further analysis.

$$(Q_{ijk} - \bar{Q}_i)t^{-1} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk} \quad (2)$$

where Q_{ijk} is the compound content at the end of the experiment (μg g^{-1}) for the soil type i, the treatment j (worms, without worms), and container k; $\bar{Q}_i$ is the mean compound content at the beginning of the experiment (μg g^{-1}) for the soil type i, and t is the duration of the experiment (days); μ is the mean release/accumulation rate (μg g^{-1} d^{-1}), α_i is the effect of the soil type i, β_j is the effect of the treatment j, $\alpha\beta_{ij}$ is the interaction between soil type i and treatment j, and e_{ijk} is the residual term. The analysis was conducted with weighed residuals as in the case of the previous analysis (*see* Eq. 1). Multiple comparisons with a least significant difference (LSD) method were used to test the differences between the soil types and between the worm treatments separately for each soil type. The calculated release rate ($\mu + \alpha_i + \beta_j + \alpha\beta_{ij}$), reported for each soil type and treatment, is directly comparable to γ_{ij} in Eq. 1. A positive release rate refers to an increasing compound pool size.

Normality of the data and the homogeneity of the variances were checked graphically (Q-Q plots, scatter plots). Differences in the soil characteristics between the soil types were tested with Tukey's test. Differences at the $p < 0.05$ level were considered significant in all analyses. Statistical analyses were performed using SPSS ver. 20.

Results

In the experiment, soil type controlled the N release more than the worm treatment (Table 3). There was an interaction between soil type and worm treatment only in $NO_3^--N_{sol}$ and $NH_4^+-N_{ex}$. The largest amount of $NH_4^+-N_{sol}$ was released from mor (soil types 1 and 2 in Fig. 3) in the presence of enchytraeids. In these soils, as well as in the highly decomposed peat (soil types 4 and 6 in Fig. 3), ca. 80% of total dissolved N was released in the form of $NH_4^+-N_{sol}$. In mor without worms and in slightly decomposed peat with and without worms (soil types 3 and 5 in Fig. 3), the release was mainly in the form of DON. The release rate of $NO_3^--N_{sol}$ was negligible as compared with other N compounds (Fig. 3).

$NH_4^+-N_{ex}$ mostly increased, i.e. the release rate was positive or near zero, whereas ON_{ex} decreased in all soil types during the incubation (Fig. 4). As a whole, the estimated rate of $NH_4^+-N_{ex}$ release was an order of magnitude higher than the release rate of $NH_4^+-N_{sol}$, except for mor without worms and slightly decomposed peat with and without worms, where no net release of

Table 3. The effect of the soil type, the worm treatment, and their interaction in the models (Eqs. 1 and 2).

	Soil	Worm	Soil × worm
$NH_4^+-N_{sol}$*	$F_{5,40} = 39.42, p < 0.0001$	$F_{1,51} = 5.78, p = 0.02$	$F_{5,40} = 1.99, p = 0.101$
$NO_3^--N_{sol}$*	$F_{5,40} = 9.28, p < 0.0001$	$F_{1,51} = 1.49, p = 0.228$	$F_{5,40} = 2.74, p = 0.032$
DON*	$F_{5,35} = 40.10, p < 0.0001$	$F_{1,37} = 0.36, p = 0.552$	$F_{5,35} = 1.40, p = 0.248$
LMW-DON*	$F_{5,41} = 23.59, p < 0.0001$	$F_{1,47} = 2.55, p = 0.117$	$F_{5,41} = 1.57, p = 0.189$
$NH_4^+-N_{ex}$**	$F_{5,36} = 37.96, p < 0.0001$	$F_{1,36} = 14.43, p = 0.001$	$F_{5,36} = 4.83, p = 0.002$
ON_{ex}**	$F_{5,36} = 149.00, p < 0.0001$	$F_{1,36} = 0.16, p = 0.693$	$F_{5,36} = 1.39, p = 0.251$
N_{mic}**	$F_{5,36} = 17.48, p < 0.0001$	$F_{1,36} = 0.01, p = 0.905$	$F_{5,36} = 1.11, p = 0.371$

* Eq. 1, ** Eq. 2.
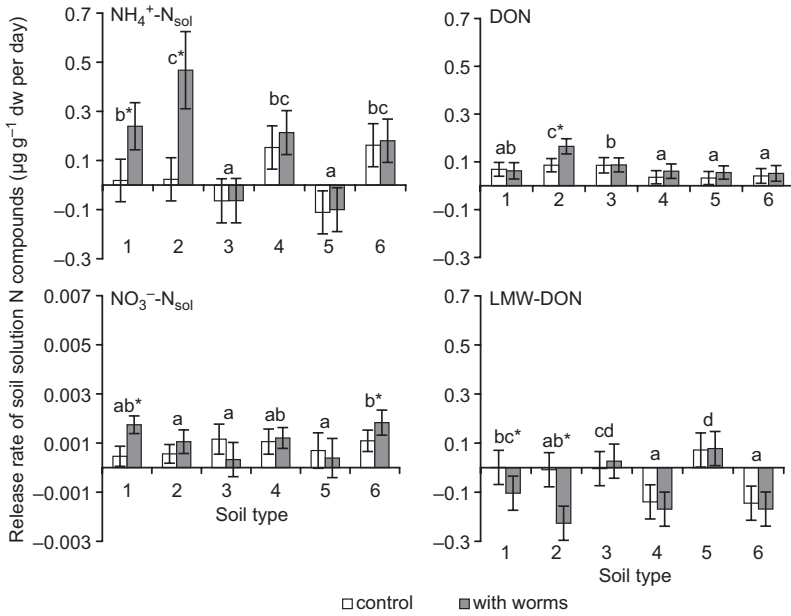
Fig. 3. Calculated release rates of soil solution N compounds, with 95% confidence intervals ($n = 4$). When the confidence interval bar intersects the x-axis, the release rate does not differ from zero. The letters a–e above the columns denote statistical differences (or their lack) between the soil types, and asterisks (*) indicate significant effect of worms on the release rate (*a priori* contrasts). Soil types: (1) mor, medium fertility, (2) mor, low fertility, (3) *Carex–Sphagnum* peat, slightly decomposed, (4) *Carex–Sphagnum* peat, highly decomposed, (5) *Sphagnum* peat, slightly decomposed, (6) *Sphagnum* peat, highly decomposed.
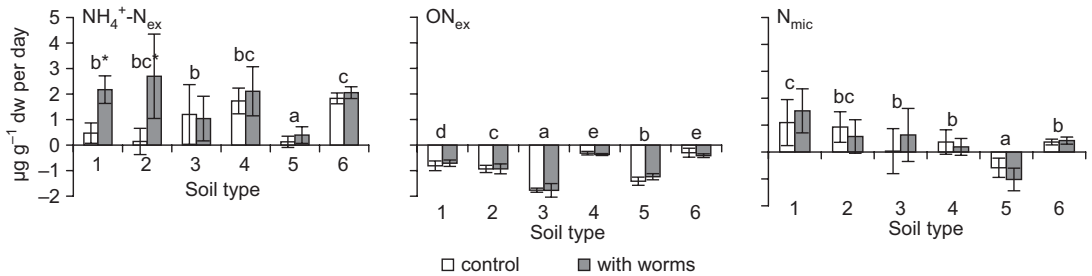
Fig. 4. Calculated release rates of extractable N compounds and the accumulation rate of microbial biomass N, (± 95% confidence intervals, $n = 4$). When the confidence interval bar intersects the x-axis, the release rate does not differ from zero. Over the columns, the letters a–e denote statistical differences (or their lack) between the soil types and asterisk (*) is shown for significant effect of worms on the release rate (post-hoc LSD). Treatments: control (without worms) and with worms. Soil types: (1) mor, medium fertility, (2) mor, low fertility, (3) *Carex–Sphagnum* peat, slightly decomposed, (4) *Carex–Sphagnum* peat, highly decomposed, (5) *Sphagnum* peat, slightly decomposed, (6) *Sphagnum* peat, highly decomposed.

NH_4^+-N_{sol} was detected. The two highly decomposed peats (soil types 4 and 6 in Figs. 3 and 4) showed statistically similar release rates for all N compounds, while the N release rates between the two mor types and between the two slightly decomposed peats differed (Figs. 3 and 4).

At the beginning of the experiment, ca. 90% of TN_{ex} was in the form of ON_{ex} in all soil types

(Table 2). ON_{ex} pool was the largest in slightly decomposed peat and the smallest in highly decomposed peat. The initial DON pool was larger in peat than in mor, but only in highly decomposed peats the DON content was higher than the NH_4^+-N_{sol} content (Table 2). In other soil types, NH_4^+-N_{sol} was the dominant fraction of the dissolved N. The proportion of dissolved N

in extractable N pool was higher in peat (10%–22%) than in mor (3%–6%) (Table 2).

The comparison of the worm treatments separately for each soil type showed that enchytraeids enhanced the release of NH_4^+-N_{sol} and NH_4^+-N_{ex} in mor, as in the absence of worms there was practically no net mineralization of N (Figs. 3 and 4). The enhanced mineralization of N is also reflected in the LMW-DON pool which decreased in the presence of worms in mor. The worms increased the DON release only in low-fertility mor (soil type 2 in Fig. 3). In general, released DON was predominantly in the form of HMW-DON as the LMW-DON pool decreased or remained unchanged during the experiment, with the exception of slightly decomposed *Sphagnum* peat (soil type 5 in Fig. 3), where the LMW-DON pool increased. No effect of the worms on N_{mic} was found. Microbes comprised initially ca. 2%–4% of the total N content in mor and slightly decomposed peat and 0.3% in highly decomposed peat. N_{mic} increased or remained unchanged in all studied soil types except in slightly decomposed *Sphagnum* peat, where N_{mic} decreased. During the incubation, the number of enchytraeids in mor increased from about 8000 indiv. m^{-2} to over 70 000 indiv. m^{-2} (Table 2). Reproduction of enchytraeids, as estimated by the number of individuals, was lower in peat than in mor. Defaunation before the incubation was successful since no enchytraeids were found in the control containers.

Discussion

Impact of soil type and enchytraeids on N release

We quantified the potential net release rates of organic and mineral N fractions in the decomposition of boreal organic matter. The measured N release rates can be converted into decomposition model parameters as shown by Laurén *et al.* (2012). An advantage of the laboratory experiment was that it enabled us to control the environmental conditions and simplify the system by exclusion of some processes and focusing on the remaining ones. The missing vegetation and microbe–vegetation interactions, as well as the missing seasonal variability in environmental conditions mean that the results are not directly comparable to field measurements. However, simplification of the system is necessary from the modelling point of view. Laurén *et al.* (2005) modelled water and nitrogen processes and their response to clear-cutting in a forested catchment and produced a detailed simulation of N balance components in control and clear-cut areas. Their results showed how released N in nutrient-limited conditions was mostly bound in soil and vegetation with minor leaching losses occurring through export with water flow. The largest and clearly dominant components of N balance were decomposition, plant uptake (by trees and ground vegetation) and immobilization. The results of Laurén *et al.* (2005) demonstrated that the validation of the modelled N balance against merely N export data without measurements of the largest N fluxes contains large uncertainties. Improvements in model development and validation call for data that support separate assessment and quantification of the main N fluxes.

Soil type was the major factor controlling the N release in the experiment. The differences between the soil types were largest in N mineralization, while the differences in DON release were relatively small. As expected, the release rates of DON and ON_{ex} were smallest in highly decomposed peats. It is well established that litter quality controls the decomposition rate of organic matter (Prescott 2010), and that the decomposition of *Sphagnum* peat is slow due to the chemical composition of litter and excessively high water content in peat (Johnson and Damman 1993). The degree of decomposition may explain the opposite NH_4^+-N_{sol} changes between slightly and highly decomposed peats. The slightly decomposed peat had clearly larger ON_{ex} pool than the highly decomposed peat. ON_{ex} pool consists of complex molecules with high sorption capacity (Stevenson and Cole 1999), and therefore NH_4^+-N_{sol} in slightly decomposed peat can be effectively immobilized by sorption. The positive rate of NH_4^+-N_{ex} release in slightly decomposed peat indicates that N mineralization took also place in slightly decomposed peat, but that the released NH_4^+-N was adsorbed on organic matter.

We found the highest rate of N release and microbial N accumulation in the two mor types

incubated with worms. The microbes did not compete with vegetation for N resources, which probably enabled the accumulation of N_{mic} in mor. The rate of $NH_4^+-N_{sol}$ release from mor with worms was close to that reportade by Park *et al.* (2002), who incubated sieved temperate deciduous forest floor material at +15 °C. The release of DON in the current study was about 10% or less as compared with the results of Park *et al.* (2002). In the absence of worms, the high mineralization rate of N in highly decomposed peat may be due to the high initial DON content and low accumulation of N into microbial biomass. The initial N_{mic} in all soil types was within the range of 0.5%–6% found in the organic layer of Finnish coniferous forest soils and drained peatlands (Smolander *et al.* 1994, Potila and Sarjala 2004).

The results show that in the decomposition of slightly decomposed peat, DON is the dominant fraction of the released dissolved N. Furthermore, the initial pool of DON and the proportion of dissolved N in the total extractable N pool were larger in peat than in mor. The large DON pool is probably connected to high water content. The results, together with a high storage of organic matter in peatlands, suggests that more N is susceptible for leaching from peatlands than from upland soils, which supports the findings of Kortelainen *et al.* (2006) and Sarkkola *et al.* (2012) who reported the leaching of N to be greater in peatland than in upland dominated catchments.

We found a positive effect of enchytraeids on the mineralization of N in mor, but the ON_{ex} pool was not affected by the presence of enchytraeids. This indicates that enchytraeids enhanced mineralization from sources other than ON_{ex} pool, e.g. by excreting N-rich compounds (Didden 1993) and processing dead organic matter into a more available form for microbes (Cole *et al.* 2000). The worms did not affect microbial-biomass N. Hedlund and Augustsson (1995) found that depending on the intensity of grazing, enchytraeids either increase or decrease the microbial biomass. The increase is enabled by both stimulated activity of decomposer microbes and by enhanced dispersal of fungal propagules by the worms. Previous studies have shown that enchytraeids (*C. sphagnetorum*) can increase mineralization of N and C, as well as the release

of dissolved organic matter (Cole 2000, 2002, van Vliet *et al.* 2004, Laurén *et al.* 2012), but the effect of enchytraeids on the N dynamics is dependent on environmental conditions and resource quality (Haimi and Siira-Pietikäinen 2003).

We expected worms to be influential not only in mor but also in slightly decomposed peat as the latter substrate should include resources for decomposer microbes as well. In peat, the population of worms at the end of the experiment was clearly smaller than in mor, which may explain the minor effect of worms on the N release in peat. It is possible that the amount of the introduced worms was not sufficient, or as the enchytraeids originated from mor, the change in habitat may have slowed down their reproduction. However, the increased enchytraeid numbers as compared with the introduced numbers in peat show that the worms were able to reproduce also in peat. The density of worms corresponds to the densities found in nature. In the mor and peat soils, the worm density — most of them living close to the soil surface — varies from a few thousands up to more than 100 000 indiv. m^{-2} (> 1 g dw m^{-2}) (Didden 1993, Räty and Huhta 2004).

N balance during the incubation

In our study, the quantitative changes in the extractable N pools were substantially greater than the N-pool changes in soil solution, indicating large changes in the adsorbed N pool. The increase in $NH_4^+-N_{ex}$ was large as compared with the increase in $NH_4^+-N_{sol}$, suggesting that most of the released NH_4^+-N was adsorbed to soil particles or on colonies of microorganisms attached to the particle surfaces. In contrast, the ON_{ex} pool decreased despite the increasing DON pool. Laurén *et al.* (2012) found similar trends of the decreasing extractable organic N pool and the increasing NH_4^+-N pool when incubating mor samples. The decrease in ON_{ex} is most likely explained by mineralization; the main part of the decreasing ON_{ex} appears to be transformed into $NH_4^+-N_{ex}$. Gaseous N fluxes through denitrification have a negligible role in the soil N mass balance in acid, nitrate-poor boreal forest soils

(Regina *et al*. 1996, Paavolainen and Smolander 1998). Therefore, the soil N mass balance was mostly controlled by the decomposition, immobilization, and solute transport processes.

It is likely that defaunation of the soils prior to the start of the experiment brought about enhanced proportions of NH_4^+-N_{sol} in the soils. It is well established that substantial amounts of NH_4^+-N_{sol} are released from dead microbes and other biomass after freezing and thawing of the soil (Huhta *et al*. 1989). Another factor affecting the NH_4^+-N_{sol} contents at the beginning of the incubation can be the soil sampling which was conducted in early summer. Piirainen *et al*. (1998) found that NH_4^+-N flux from the O horizon in Kangasvaara sites was highest in the spring during snow melt, and that the flux of organic N increased during the summer. At the end of the incubation, DON was the dominant form of dissolved N in mor without worms and in slightly decomposed *Sphagnum* peat, but in other samples NH_4^+-N_{sol} dominated and the proportion of NH_4^+-N_{ex} in the total N_{ex} pool was increased in all soils. The lack of vegetation may have led to accumulation of NH_4^+-N. Vegetation, through root exudates, is also considered the dominant source of LMW compounds (Fischer *et al*. 2007) and therefore, together with the rapid turnover of the LMW-DON pool (e.g. Kalbitz *et al*. 2003a, Kiikkilä *et al*. 2006), the low amounts of LMW-DON were expected. It is also highly likely that root exudates stimulate growth and activity of soil microbes and that enchytraeids, being partially microbial feeders, may benefit from the increased food resources, thereby further enhancing decomposition and nutrient mineralization. Microbial utilization of labile LMW fraction in the current experiment probably caused accumulation of the refractory HMW fraction. Kalbitz *et al*. (2003b) and Hagedorn and Machwitz (2007) showed that the refractory compounds of dissolved organic matter accumulate during biodegradation.

The partitioning of organic N release between the labile and refractory fractions deserves further attention as a subject of research. It is likely affected by vegetation and by soil temperature and water content, and therefore the abiotic controls of the decomposition in the presence of vegetation are subjects of a further study. The current study showed that the changes in the adsorbed N pool were larger than the changes in the soil-solution N pool, and thus the changes in both pools should be monitored in the future studies to cover the full N dynamics in soil, and acquire information for solute transport modeling e.g., to detect whether an equilibrium or kinetic sorption process description is needed in the solute transport model and whether biodegradation occurs at similar or dissimilar rates for dissolved and adsorbed N forms.

In conclusion, our results suggest that greater N leaching in peatlands than in upland soils is partly due to the larger proportion of N in the dissolved form in peat than in mor. The partitioning of dissolved N release between NH_4^+-N_{sol} and DON was affected by the soil type. The relative DON release was higher from peat than from mor, and higher from slightly decomposed peat than from highly decomposed peat. We found that enchytraeids enhanced mineralization of N. If the population density changes in the future, e.g. due to climate change, it will affect the N dynamics in the soil. Because different types of mor and peat typically exist in boreal forested catchments, N release rates for the two types of mor and the four types of peat will provide a more realistic description of decomposition outputs. This will benefit solute transport modelling, and enable upscaling of the results from a soil-sample scale into hillslope and catchment scales.

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