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Causes for the small scale variability of nitrate concentration in seepage water of an N saturated mature spruce stand

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Abstract

• **Context** In N-saturated forests nitrate concentrations in seepage water ($\text{NO}_3^-_{\text{seepage}}$) regularly show high spatial variability even within homogeneous stands. Up to now the reasons of this variability are not fully understood.
• **Aims** The main objective was to identify the crucial parameters that control spatial variability of $\text{NO}_3^-_{\text{seepage}}$ at the Höglwald site.
• **Methods** We investigated a multitude of parameters (e.g. N turnover, root biomass, soil chemistry, soil physics, stand parameters) and related them to $\text{NO}_3^-_{\text{seepage}}$, measured in 40 cm depth with suction cups.
• **Results** A small number of biological parameters (net N mineralization, root distribution, and stand density) explained

up to 93 % of the variability of $\text{NO}_3^-_{\text{seepage}}$ in linear regression models. Net N-mineralization rates in the humus layer and fine root biomass in the upper mineral soil influenced $\text{NO}_3^-_{\text{seepage}}$ positively. Fine root biomass in deeper soil layers (30–40 cm depth) and stand density had a negative influence.

• **Conclusion** The rate of net N mineralization in the organic layer is decisive for the nitrate production in the soil. Roots in the upper mineral soil increase $\text{NO}_3^-_{\text{seepage}}$ by intensive water uptake but excluding nitrate at the same time. The variation of these two parameters is responsible for most of the small-scale variability of $\text{NO}_3^-_{\text{seepage}}$.

Keywords Nitrate leaching · Spatial variability · N turnover · Net N mineralization · Höglwald · N saturation · Fine root biomass

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Contribution of the co-authors Michael Kohlpaintner: all field and laboratory measurements except nitrification and mineralisation rates, which were made by Boris Matejek. Michael Kohlpaintner and Axel Göttlein: running the data analysis. Michael Kohlpaintner and Christian Huber: interpretation of the data and writing the paper. Christian Huber and Axel Göttlein: supervision of the work and coordination of the project.

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1 Introduction

Due to persisting high levels of N deposition to forest ecosystems, NO_3^- leaching has become a serious problem in large parts of central European forests (Dise et al. 1998; Kreutzer 1995; Gundersen et al. 2006). In the last decades, many forests shifted from a status of N limitation to N saturation, which is indicated by the onset of net nitrification and enhanced NO_3^- leaching (Aber et al. 1989; Vitousek et al. 1997). An input of about $10 \text{ kg N year}^{-1}$ by throughfall precipitation is considered to be a threshold for elevated NO_3^- concentrations in seepage water (Gundersen et al. 1998; Dise et al. 1998; Kristensen et al. 2004). This threshold is exceeded by far for most German forests (Gauger et al. 2008). Mellert et al. (2005) found in a comprehensive survey in Bavaria in the years 2001 and 2002 that 37 % of the investigated forests show clear signs of N saturation.

NO_3^- concentrations in seepage water (hereafter referred to as $\text{NO}_{3\text{seepage}}^-$) show high spatial variability in N saturated forests, even in homogeneous even aged forest stands (Mellert et al. 2008; Kohlpaintner et al. 2009; Gundersen et al. 2006; Manderscheid and Matzner 1995a; Huber et al. 2004). The high spatial variability makes it difficult to estimate $\text{NO}_{3\text{seepage}}^-$ with an adequate precision (Kohlpaintner et al. 2009; Manderscheid and Matzner 1995a).

Due to its importance for forests and adjacent ecosystems, NO_3^- leaching has been investigated in many studies (Gundersen et al. 2006). However, the causes for the observed variability still remain quite unclear (Manderscheid and Matzner 1995b; Kohlpaintner et al. 2009; Mellert et al. 2008). Most studies investigated only a few major parameters concerning nitrate variability, like N input (Dise et al. 1998), tree species composition (Rothe et al. 2002), stand age (Rothe and Mellert 2004), and forest management (Huber et al. 2004), but a comprehensive understanding about the regulating factors is still missing (Dise et al. 2009).

The homogeneous Norway spruce stand at the N saturated “Höglwald” site has shown very high spatial variability in nitrate (Kohlpaintner et al. 2009; Huber et al. 2010). At that site, Kohlpaintner et al. (2009) could explain about 40 % of the variability of $\text{NO}_{3\text{seepage}}^-$ with easy and nondestructive measurements in an intensive campaign with 121 suction cups in a mature spruce stand. The majority of the variability could be explained by vegetation coverage and stand density. Within the same site, Matejek et al. (2008) selected 20 measuring places and found that net N mineralization in the organic layer explains about 50 % of the variation of $\text{NO}_{3\text{seepage}}^-$. At the same 20 spots, we measured a multitude of additional soil physical, chemical, and biological parameters, which may influence NO_3^- leaching. Compared to Matejek et al. (2008) who only investigated the organic layer of the soil, we additionally studied the mineral soil down to 40 cm in a 10 cm depth resolution. The main objective of these measurements was to identify more parameters that may control nitrate production and leaching and to develop a statistical model, which ideally explains a large part of the detected spatial variability of $\text{NO}_{3\text{seepage}}^-$ at the investigated site. Therefore, in this paper, we compiled all data obtained in this study as well as the data about microbiological N turnover in the organic layer presented in Matejek et al. (2008) and tested their effects on nitrate concentration in seepage water. We first investigated the relationship of each measured parameter to $\text{NO}_{3\text{seepage}}^-$. In a second step, we developed a multivariate statistical model to explain as much variability of $\text{NO}_{3\text{seepage}}^-$ as possible.

2 Material and methods

2.1 Site description

The “Höglwald” long-term ecological monitoring and experimentation site is located about 50 km west of Munich (48°17' N; 11°04' E) at an elevation of 540 m asl. The mean annual bulk precipitation between the years 1985 and 2004 was 947 mm, and the mean annual temperature was 7.9°C (Huber et al. 2010). In the year of the investigation (2005), bulk precipitation was 1,050 mm and mean temperature was 8.0°C. The region around the Höglwald is characterised by a mix of relatively small coniferous forests and intensive agriculture. The site is N saturated according to the definitions of Gundersen et al. (2006) and Aber et al. (1989). The annual N input via throughfall is about 30 kg ha⁻¹ year⁻¹, while output with seepage water is about the same amount (Rothe et al. 2002; Huber et al. 2010). The soil is an Alisol according to the FAO classification or a Parabraunerde according to the German soil classification. It is derived from Pleistocene loess over tertiary silty sand. The topsoil is strongly acidified with a base saturation of <10 % in 40 cm depth and a pH of 3.7 (KCl). The mineral soil is covered by an organic layer (6–8 cm thick, typical moder). In the organic layer, the base saturation of the cation exchange capacity is rather high (80 % in the litter layer, 40 % in the O_h). However, pH values are extremely low with a minimum of 2.75 (KCl) in the O_h horizon. N content in the organic layer is about 1.7 % with a C/N ratio of 23–27. Soil texture ranges from loamy sand to sandy loam. No coarse material (>2 mm) is present in the mineral soil. A detailed description of the Höglwald experimental site is given in Kreutzer and Weiss (1998). The newly installed area for this NO_3^- study is located about 800 m in the south east of the “control plot” A1 of the “Höglwald” site. More information about the investigated site can be found in Kohlpaintner et al. (2009).

2.2 Experimental setup

In April 2005, 121 suction cups (tension ceramic lysimeters, SKL 100, UMS GmbH München) were installed vertically in hand-augered holes in a 2 × 2 m grid in 40 cm depth (from the surface of the mineral soil). Implementation was done carefully according to a standard procedure with a minimum of disturbance. No organic material was displaced into the mineral soil. A rubber collar placed around the shaft at the level of the mineral soil prevented preferential flow along the tubing of the suction cup. After discarding the first sample (at least 200 ml per suction cup), soil solution was collected at least monthly between May and October 2005. A vacuum suction of 60 kPa was applied 1 week before the date of sampling. In the field, the glass bottles for soil water

collection were covered by plastic buckets to reduce light- and temperature-induced element turnover. After the installation, the whole sampling area was protected by a fence. For more details, see Kohlpaintner et al. (2009). In July 2005 (after four samplings), 20 suction cups were selected, which covered the entire previously detected concentration range of NO_3^- from 1 to 160 mg l^{-1} (Kohlpaintner et al. 2009).

At these 20 places, we measured a multitude of physical, chemical, biological, and stand parameters thought to influence NO_3^- leaching. Table 1 gives an overview of the measured parameters, the soil layers where they have been determined, and the date or time span when measurements took place.

From July to October, we measured water and N input via throughfall with small samplers (area=50.3 cm^2) directly above the suction cups. The small sampling area was chosen in order to not disturb water and ion input to the measuring spots. The depth of the organic layer was measured during the

implementation of the suction cups in April and during soil sampling in October. In addition, the total dry weight of the organic layer was determined.

From the middle of July to the middle of September, Matejek et al. (2008) measured net N mineralization and nitrification rates with the buried bag technique (Hart et al. 1994) as well as gross rates of nitrification using the barometric process separation method (Ingwersen et al. 1999) and extractable amounts of nitrate (NO_3^-) and ammonium (NH_4^+) in the organic layer. The incubation time for the determination of N turnover rates was 4 weeks. Although Matejek et al. (2008) used area related N turnover rates (e.g., milligram of N per day per square meter) already including substrate availability at the different sampling spots, in this paper, we refer to dry weight of organic matter (e.g., milligram of N per day per kilogram) and considered the substrate availability separately as weight and/or depth of the organic layer in kilogram per square meter and/or centimeter, respectively.

Table 1 Investigated parameters that may influence NO_3^- seepage with measurement depth/layer and time/period

Parameter group	Parameter	Unit	Layer ^a	Time/period of measurement	Number of variables
Nitrat in seepage water	NO_3^-	mg l^{-1}	4	April–October	
N input via throughfall	Input of NH_4^+ , NO_3^- and total inorganic N	mg m^{-2}	-1	July–October	3
	Average volume weighted concentration of NH_4^+ , NO_3^- and total inorganic N	mg l^{-1}	-1	July–October	3
Water input and substrate availability in the organic layer	Input of water	l m^{-2}	-1	July–October	3
	Dry weight organic layer	g dw m^{-2}	0	October	1
	Depth of organic layer	cm	0	October	1
N-turnover parameters in the organic layer	Net N mineralization, net nitrification, gross nitrification	$\text{mg N day}^{-1} \text{kg}^{-1} \text{dw}$	0	July–August	3
	Pools of NH_4^+ and NO_3^-	$\text{mg N kg}^{-1} \text{dw}$	0		2
N-uptake parameters	Tree root biomass (<2, 2–5, >5 mm)	g m^{-2}	0–4	October	15
	Vegetation cover of mosses, oxalis, other vascular plants	% coverage	-1	July	3
Chemical soil parameters	pH in H_2O and KCl		0–4	October	10
	N and C content	%	0–4	October	10
	C/N	Ratio	0–4	October	5
Physical soil parameters	Bulk density	g cm^{-3}	1–4	October	4
	Pore volume of macro- (>50 μm), meso- (50–10 μm) and fine pores (<10 μm) and whole pore volume	vol.%	1–4	October	16
	Grain size distribution	% sand, % silt, % clay	1–4	October	12
Stand parameters	Distance to next trees	cm	-1	July	1
	Breast high diameter of the next trees	cm	-1	July	1
	Basal area of trees within a radius 4 m	cm^2	-1	July	1
	Number of trees in a radius of 4 m	n	-1	July	1
Sum of variables					93

The number of variables which are finally tested with NO_3^- seepage is the result of the parameter/-s multiplied by the number of investigated soil layers dw dry weight

^a -1=above organic layer, 0=organic layer, 1=mineral soil 0–10 cm, 2=mineral soil 10–20 cm, 3=mineral soil 20–30 cm, 4=mineral soil 20–30 cm

The vegetation coverage in a circle of 60 cm around each suction cup was determined in July. Separately, we reported the percent coverage of the different vegetation layers (shrub, herbaceous, and moss layer) and major species [*Oxalis acetosella* (L.), different grasses, and tree saplings]. At this time, we also determined stand parameters by measuring the distance of the suction cups to the next trees and the breast height diameter of these trees.

At the end of the vegetation period in October 2005, the 20 measuring spots were sampled destructively to determine chemical, physical and biological parameters.

Organic layer samples were taken using a cylindrical template with a diameter of 45 cm. Tree roots were separated cautiously using a 5-mm sieve. Organic layer depth and total dry weight of the organic layer was determined. pH was measured in H₂O and KCl. Part of the material was dried and prepared for total C and total N analysis.

The mineral soil was sampled down to 40 cm depth in layers of 10 cm using a cylindrical steel template with a diameter of 36 cm, which was introduced into the soil prior to bulk soil sampling to assure constant volumes for the determination of root biomass and other parameters. Prior to bulk soil sampling from each layer, four undisturbed soil cores (stainless steel cylinders of 100 cm³) were taken for determination of bulk density and pore size distribution at 60, 300, and >300 mbar pressure. Mineral soil was sieved with a 2-mm mesh to separate living tree root biomass. pH value was measured in H₂O and KCl. An aliquot of each layer was dried and prepared for other chemical and physical analysis.

All root material was taken to the lab, washed, and separated into different root size classes (<2, 2–5, and >5 mm). Afterwards, roots were dried and weighted.

2.3 Chemical and laboratory analysis

In the laboratory, all seepage water and throughfall samples were filtered using membrane filters with a pore size of 0.45 μm (Schleicher and Schuell NC 45) and stored at 4°C in the dark until their analysis within a week after collection. NO₃⁻ was determined by ion chromatography (Dionex DX120). NH₄⁺ in throughfall was determined by the segmented flow analysis (Autoanalyzer from Skalar). NH₄⁺ was not detectable in seepage water.

C and N content was determined on ground soil samples with a CHN analyzer (Leco). pH was determined in H₂O and 1 M KCl using a soil to solution ratio of 1:2.5 applied on a weight basis for mineral soil and on a volume basis for organic layer samples.

Grain size distribution of the mineral soil was determined by a combination of sedimentation and sieving with an aliquot of 20 g of dried soil, which was dispersed completely with sodium pyrophosphate.

Pore volume at different matrix potentials was determined in pressure chambers where water saturated undisturbed soil cores were placed on ceramic plates using a glass fibre filter to assure capillary connection. Between 60 and 300 mbar of pressure was then applied. Cylinders were weighted before and after each pressure step and allowed to equilibrate. After the 300 mbar step, samples were dried at 105°C and weighted to determine bulk density. Pore volumes at 60, 300, and >300 mbar were calculated using the weight differences of the cylinders at the different steps. The pore volumes are equivalent to wide macro pores (>50 μm), small macro- and mesopores (50–10 μm) and meso- to fine pores (<10 μm) at the respective pressure steps.

2.4 Statistics

Prior to multiple regression analysis, the large set of variables was reduced. Parameters that obviously had no influence on the variation of the nitrate concentration in seepage water were excluded. Therefore, we tested each variable with the mean NO₃⁻_{seepage} of the eight samplings from April to October in a simple regression analysis. Only variables that showed *F* values above 1.5 in the ANOVA were considered for multiple regression analysis (32 variables, see Table 2). This *F* value was equivalent to an explanation power of at least 8 % of the variability of NO₃⁻_{seepage}. As many of the potential predictors were correlated with each other, we calculated stepwise linear regression models to identify the most significant multivariate relationships between NO₃⁻_{seepage} and measured parameters. This was done for every seepage water sampling date and the mean NO₃⁻_{seepage} with all 32 variables shown in Table 2. In the model, only variables were included, which contributed to explanation at a significance level of *p*<0.05. Afterwards, we took all variables that were significant in at least one of the models (variables listed in Table 3) and calculated for every sampling date and the mean NO₃⁻_{seepage} a stepwise backwards linear regression. Exclusion criterion in the backward procedure was a *p*>0.1. Table 3 shows a summary of the backward linear regression models.

The final models were assessed for multicollinearity. Variables that showed variance inflation factors above 5 were excluded as well as variables that caused a condition number above 30. A subsequent examination of the scatter plot of the standardized residuals versus the predicted values showed no systematic patterns in the final models presented in Table 3.

All statistical analyses were performed using SPSS 17.0.

Table 2 Descriptive statistics for measured parameters showing an F value above 1.5 when compared in a simple regression analysis with mean NO_3^- seepage and their correlation coefficient with mean NO_3^- seepage

Group	Parameter (layer/depth) ^a	Unit	Mean value	SD	CV (%)	r_{pearson}^c	F value ^d
Mean NO_3^- in seepage water	NO_3^-	mg l^{-1}	49.5	35.2	71		
N-turnover	Net N mineralization ^b	$\text{mg N kg}^{-1} \text{ day}^{-1}$	4.0	2.0	51	0.87**	54.60
	inorg N pool ($\text{NH}_4^+ + \text{NO}_3^-$) ^b	mg N kg^{-1}	143.2	61.9	43	0.80**	32.70
	Net nitrification ^b	$\text{mg N kg}^{-1} \text{ day}^{-1}$	2.7	1.5	54	0.65**	13.30
	NH_4^+ pool ^b	mg N kg^{-1}	45.5	42.9	94	0.62**	11.05
	NO_3^- pool ^b	mg N kg^{-1}	97.7	39.4	40	0.59**	9.25
Vegetation coverage	<i>Oxalis acetosella</i>	%	21	14	64	0.75**	22.98
	Mosses	%	72	30	41	-0.45*	4.53
	Other vascular plants	%	13	25	186	-0.30	1.72
Roots biomass	Roots <2 mm (1–3)	$\text{g m}^{-2} \text{ layer}^{-1}$	130.1	43.1	33	0.52*	6.59
	Roots 2–5 mm (2)	$\text{g m}^{-2} \text{ layer}^{-1}$	20.9	12.7	61	0.51*	6.31
	Roots <2 mm (4)	$\text{g m}^{-2} \text{ layer}^{-1}$	7.1	5.5	78	-0.51*	6.17
	Roots <2 mm (1)	$\text{g m}^{-2} \text{ layer}^{-1}$	100.1	36.1	36	0.51*	6.16
	Roots <2 mm (2)	$\text{g m}^{-2} \text{ layer}^{-1}$	21.9	11.6	53	0.35	2.45
	Roots 2–5 mm (1)	$\text{g m}^{-2} \text{ layer}^{-1}$	96.2	28.4	30	0.32	1.98
	Roots 2–5 mm (3)	$\text{g m}^{-2} \text{ layer}^{-1}$	10.9	12.7	116	-0.29	1.70
Chemical soil parameters	pH in H_2O (4)		4.3	0.11	2	-0.60**	9.95
	pH in H_2O (3)		4.3	0.12	3	-0.53*	7.20
	C/N (4)	ratio	6.2	1.03	17	-0.52*	6.70
	C content (4)	%	0.33	0.06	18	-0.44	4.24
	C content (3)	%	0.52	0.10	20	-0.44	4.11
	C/N (3)	ratio	8.6	1.34	16	-0.43	3.90
	N content (1)	%	0.21	0.04	19	0.35	2.43
	C content (0)	%	44.9	2.97	7	0.31	1.97
	C content (1)	%	3.5	0.85	24	0.30	1.70
	C/N (0)	ratio	25.9	1.12	4	0.29	1.61
Stand parameter	Basal area within a radius of 4 m	cm^2	3706	964	26	-0.39	3.19
	Basal area within a radius of 2.2–4 m	cm^2	2346	1357	58	-0.30	2.48
Physical soil parameters	Macropores (>50 μm) (3)	vol.%	11.4	3.7	32	-0.35	2.47
	Meso- and fine pores (<10 μm) (3)	vol.%	24.4	1.2	5	0.34	2.37
	Bulk density (3)	g cm^{-3}	1.46	0.07	5	0.34	2.32
Organic layer depth	Depth O_h	mm	32.5	6.5	20	0.32	2.05
	Depth $\text{O}_h + \text{O}_f$	mm	67.3	13.2	20	0.32	2.03

Arrangement was done first according to groups and second according to descending F values

SD standard deviation, CV coefficient of variance

^a (0)=organic layer, (1–4)=mineral soil layers from 0 to 10 (1), 10–20 (2), 20–30 (3) and 30–40 cm (4)

^b Data from Matejek et al. (2008)

^c Linear correlation coefficient with mean NO_3^- seepage; * $p < 0.05$, ** $p < 0.01$

^d F value for parameter in ANOVA with NO_3^- seepage

3 Results

3.1 Nitrate in seepage water

NO_3^- seepage showed mean values from 1.6 (SD, 1.4) to 127.7 (SD, 7.4) mg l^{-1} at the respective sampling places (Fig. 1). The mean NO_3^- seepage at the sampling dates slightly declined

from April (56.6 mg l^{-1}) to July (50.2 mg l^{-1}) and then decreased to 34.9 at the last sampling in October. Temporal variation expressed as coefficient of variance (CV) at the 20 sampling places ranged from 4 to 103 % with a mean value of 31 %. Spatial variation expressed as CV at the respective sampling dates ranged from 70 to 96 %. Temporal as well as spatial variability at the 20 sampling places were almost the

Table 3 Summary of the final linear regression models explaining the variability of NO_3^- in seepage water at the different sampling dates

Parameter (layer) ^a	Unit	Standardized regression coefficient (beta) of significantly included variables								
		April	May 1	May 2	June	July	August	September	October	April–October
1 Net N mineralization (0)	mg N kg ⁻¹ day ⁻¹	0.64	0.65	0.65	0.67	0.69	0.73	0.58	0.52	0.62
2 Roots <2 mm (1–3)	g m ⁻²	0.34	0.37	0.4	0.41	0.37	0.35	0.38	0.41	0.41
3 Roots <2 mm (4)	g m ⁻²					-0.17	-0.17	-0.30	-0.34	-0.15
4 Roots 2–5 mm (3)	g m ⁻²	-0.18	-0.17	-0.16	-0.14					
5 Basal area circ. 4 m	cm ³	-0.31	-0.27	-0.25	-0.22	-0.15				-0.21
6 Pore volume <10 μm (3)	vol. %	-	-	-	-					
7 pH H ₂ O (3)							-	-	-	-
8 Cover oxalis	% coverage									-
Sum beta ²		0.65	0.66	0.67	0.69	0.66	0.68	0.57	0.55	0.62
Adjusted multiple R ²		0.87	0.88	0.89	0.89	0.93	0.89	0.79	0.79	0.90

Shown are the standardized regression coefficients for each significant parameter and the sum of the squared beta values (sum beta²) and the adjusted multiple R² for the whole model

(-) parameter was originally included into the model but lead to high collinearity indexes and was therefore removed

^a(0)=organic layer, (1–4)=mineral soil layers from 0 to 10 (1), 10–20 (2), 20–30 (3), and 30–40 cm (4)

same as reported for the whole set of 121 suction cups in Kohlpaintner et al. (2009).

3.2 N mineralization and nitrification

Processes and parameters related with N turnover were highly correlated with NO_3^- in seepage water and showed high *F* values (Table 2). Net N mineralization measured between July and September showed the highest positive significant correlation with an *r* of 0.87 and showed rates between -1.2 and 6.7 mg N kg⁻¹ day⁻¹. The spatial variability was high (CV=51 %) and in the range

of the variability of NO_3^- in seepage water. The pool of inorganic N (NH_4^+ -N and NO_3^- -N) measured at the beginning of the incubation period ranged between 38 and 270 mg N kg⁻¹ and showed also a highly significant positive correlation and the second highest *F* value. If the pools of NH_4^+ and NO_3^- pool were considered separately, correlations were lower and ranges were from 9.3 to 186.4 (CV=94 %) and from 28.9 to 176.2 (CV=40 %) mg N kg⁻¹, respectively. Net nitrification had also a highly significant *r* and showed rates between 0.5 and 5.2 (CV=54 %) mg N kg⁻¹ day⁻¹. Gross nitrification rates in contrast showed an *F* value far below 1.5 and were not correlated with NO_3^- in seepage water (Matejek et al. 2008).

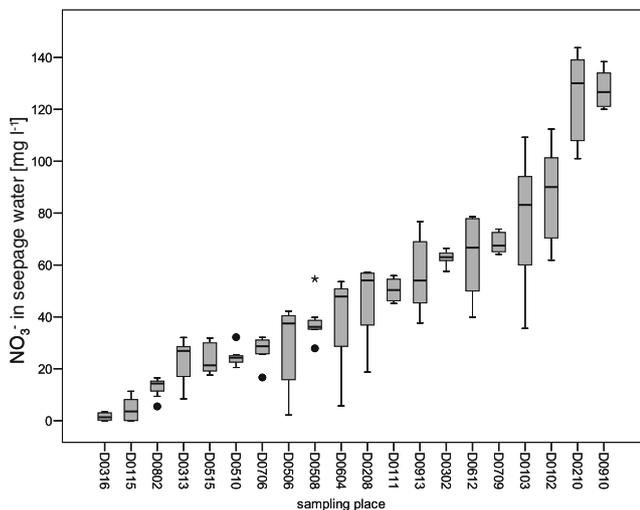


Fig. 1 Nitrate concentrations in seepage water at 40 cm depth at the different sampling places arranged in ascending mean concentrations. Every box plot contains the eight measurements from April to October at one of the 20 intensively investigated suction cups

3.3 Ground vegetation coverage

Ground vegetation coverage of *O. acetosella* (L.) ranged from 3 to 50 % and exhibited a highly significant positive correlation with the mean NO_3^- in seepage water. Coverage of mosses ranged from 2 to 98 % and showed a significant negative correlation while the coverage of all other vascular plants (range from 0 to 87 %) showed *F* values above 1.5 but was not significantly correlated with NO_3^- in seepage water.

3.4 Tree roots

Figure 2 shows the mean vertical distribution of fine and medium sized roots at the 20 sampling places. Fine root biomass (<2 mm) and medium sized root biomass (2–5 mm) in the organic layer showed no significant correlation with NO_3^- in seepage water and had very low *F* values. In contrast, fine root biomass in the upper two mineral soil layers showed *F* values

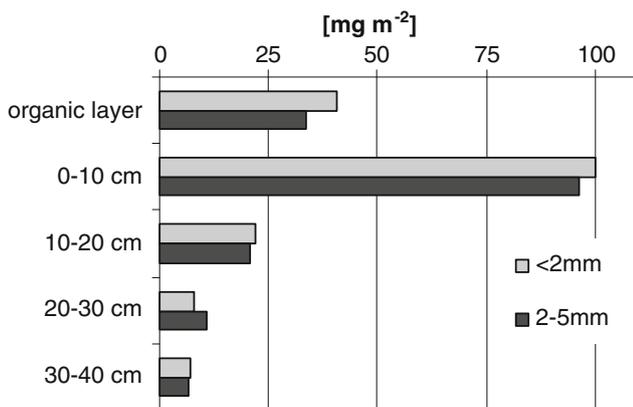


Fig. 2 Vertical distribution of fine (<2 mm) and medium-sized (2–5 mm) root biomass presented as mean values of the 20 sampling places

above 1.5. Correlations with $\text{NO}_3^-_{\text{seepage}}$ were positive and significant for fine root biomass in 0–10 cm and medium sized root biomass in 10–20 cm. For the 20–30 cm layer, only the medium sized root biomass showed an F value above 1.5 but the correlations were not significant. For the fourth layer (30–40 cm), fine root biomass showed a significant negative correlation, while medium-sized root biomass exhibited no correlation with $\text{NO}_3^-_{\text{seepage}}$ (Table 2). More than 70 % of the fine and medium sized root biomass in the investigated soil profiles was located in the organic layer and the mineral soil from 0 to 10 cm; 56 and 45 % of roots were located in the 0–10 cm layer, while fractions of fine and medium roots in 40 cm depth are below 5 % (see also Fig. 2).

3.5 Chemical and physical soil parameters

High F values and significant negative correlations were evidenced for pH in H_2O in the soil layers from 20 to 40 cm and for the C/N ratio in the fourth soil layer with pH ranges from 4.0 to 4.5. No significant correlations but F values above 1.5 were found for C contents in soil layers 3 and 4 and for the C/N ratio in the third layer as well as for C and N parameters in the organic layer and the first mineral soil layer. The pH, C, and N values of the other investigated soil layers had no F values above 1.5 and showed no relationship with $\text{NO}_3^-_{\text{seepage}}$.

Volume of macropores (>50 μm) in 20–30 cm depth, the layer directly above the sampling depth, showed F values above 1.5 as did the volume of meso- and fine pores (<10 μm) and bulk density of the same soil layer, but correlations were not significant. Pore volume and bulk density in the other mineral soil layers as well as grain size distribution in all mineral soil layers had no F values above 1.5.

Clay (particle size <2 μm) content in the different layers was between 10 and 12 %, while silt content was 30 % and sand content was 60 % with mostly fine sand.

Thickness of the O_h layer and the whole O layer ($\text{O}_f + \text{O}_h$) were not significantly correlated with $\text{NO}_3^-_{\text{seepage}}$ but showed F values around 2. They ranged from 18 to 44 mm and 29 to 90 mm, respectively, and had a CV of 20 %. The dry weight of the organic layer, which ranged from 2.0 to 10.1 kg m^{-2} (mean=6.5 kg m^{-2}), had a CV of 30 % and showed no F values above 1.5.

3.6 Stand parameters

Basal area of spruce trees in a circumference of 4 m around the suction cup, which can be interpreted as a measure of stand density ranged from 2,200 to 5,500 cm^2 . It was not correlated with $\text{NO}_3^-_{\text{seepage}}$ as was the basal area between 2 and 4 m around the suction cup but showed F values above 1.5.

3.7 Throughfall

Water input via throughfall from mid-July to mid-October ranged from 159 to 233 lm^{-2} . Mean value was 187 lm^{-2} , and the CV was 10 %. N input via throughfall ranged from 3.8 to 9.1 kg Nha^{-1} at the 20 sampling places (mid-July to mid-October) with a mean value of 6.1 kg Nha^{-1} . Neither throughfall inputs of NH_4^+ and NO_3^- nor the volume weighted concentrations of these parameters showed correlations with the mean $\text{NO}_3^-_{\text{seepage}}$ (F values below 1.5) and were therefore not used for linear regression analysis. CV for N input parameters ranged from 23 to 29 %.

3.8 Regression models

Multiple R^2 were high and the models explained between 78 and 93 % of the variation of $\text{NO}_3^-_{\text{seepage}}$. Net N mineralization was included in all models and had a positive effect on $\text{NO}_3^-_{\text{seepage}}$. Influence was stronger from April to August and declined in September and October, but it always explained the highest part of the variation in the models. Sum of the squared standardized regression coefficients (β^2) are consistently lower compared with adjusted multiple R^2 (Table 3).

Fine root biomass in the mineral soil from 0 to 30 cm depth was also included in all of the models and had a positive influence on $\text{NO}_3^-_{\text{seepage}}$. In contrast fine roots in 30–40 cm depth had no significant explanatory influence until July where it started to increase negatively until October. Medium-sized roots in 20–30 cm depth had a small negative influence from April to June but none afterwards. Basal area of the trees showed a significant negative influence on $\text{NO}_3^-_{\text{seepage}}$, which declined from April to July but was not significant from August and October. In addition, volume of pores <10 μm and pH in 20–30 cm depth had a significant influence on $\text{NO}_3^-_{\text{seepage}}$ from April to June and

from August to October, respectively. However, both variables were excluded from the models due to high collinearity indexes. *O. acetosella* (L.) exhibited only in the model with the mean NO_3^- seepage a significant positive influence but was also excluded because of collinearity.

4 Discussion

Studies investigating the causes for spatial patterns of NO_3^- seepage were mostly focused on one or few parameters (Manderscheid and Matzner 1995a; Matejek et al. 2008; Kohlpaintner et al. 2009; Mellert et al. 2008). However, the processes and parameters involved in small-scale N leaching at the stand level are supposed to be complex and may involve multiple factors. However, the results of the linear regression models show that relatively few parameters can explain up to 93 % of the observed variability of NO_3^- seepage at our site. In all of the models, net N mineralization and fine root biomass in the upper mineral soil are positively influencing nitrate concentration, while roots in deeper soil layers mostly had a negative effect on nitrate. In addition, basal area of spruce trees near the sampling places is included in most of the models and exhibited a negative effect on nitrate. The plausibility of these factors on the nitrate concentration in seepage water will be discussed.

4.1 N turnover

Net N mineralization measured from July to August in incubation periods of 4 weeks showed highest correlation with NO_3^- seepage and explained most of its small-scale variability at all sampling dates from April to October (see also Matejek et al. 2008). It is surprising that, even for the seepage water samplings from April to June (up to 3 months before the start of the incubations), the explanation power of net N mineralization was high. Even for the rates measured in July the downward transport of the produced N compounds with seepage water will take weeks to months depending on water fluxes with seepage. This implies that the rates of net N mineralization or at least their ratios may be relatively constant over a longer time period. At the end of the vegetation period, NO_3^- seepage declined from 50 mg l^{-1} in July to 35 mg l^{-1} in October. Furthermore, the explanation power of the models decreased for this period. Both findings may be caused by changes in net N mineralization rates and indicates a temporal variability in this process, which was not investigated in this survey. Laverman et al. (2000) found considerable temporal variation in net N mineralization rates during 1 year of investigation in an N saturated Scots pine forest in The Netherlands. The spatial variability of net N mineralization in the organic layer at the

investigation site was partly explained (~60 %) by a linear regression model, which includes soil water content and amount of substrate (Matejek et al. 2008)

The sum of the inorganic N pool (NH_4^+ and NO_3^-) showed a high positive correlation with NO_3^- seepage, which is reasonable as the pool of inorganic N is, beside throughfall input, the result of the mineralization activity. In contrast to this, the correlations between NO_3^- seepage and other N turnover rates, as well as the pools of NH_4^+ and NO_3^- when considered separately, were considerably lower. Due to the high intercorrelations with net N mineralization, these parameters were not included into the linear regression models.

It may be surprising that net nitrification explains less variability than net N mineralization. However, there are some inevitable methodological side effects, which may help to explain this result. Nitrification rates were determined under the exclusion of tree/plant root uptake, but according to our flux calculations from other plots, the N uptake occurs in the forest floor mainly via uptake of NH_4^+ (Gessler et al. 1998; Kronzucker et al. 1996). Therefore, in nitrification experiments, the competition between microorganisms and plant roots is inevitably eliminated. Hence, more substrate is available for nitrification in the experiment than would be under natural conditions. We assume that the potential for net N mineralization is more important for nitrate variability than the potential to nitrify at a certain measuring spot, as in reality a large part of ammonified substrate would be taken up by the tree roots before nitrification could occur. The higher correlation of NO_3^- seepage with the sum of inorganic N pools in the organic layer compared to the separated pools (see Table 2) supports this hypothesis.

This suggests that conversion rates of organic N into inorganic N in the organic layer are a very important driver for the observed variability of NO_3^- seepage. Another aspect is that part of the NH_4^+ produced in the humus layer is leached into the mineral soil where it may be immobilized, taken up or nitrified. This NO_3^- resulting from nitrification in deeper soil layers may influence NO_3^- seepage. As we measured in situ nitrification only in the organic layer, we have no information about this parameter in the mineral soil. However, laboratory measurements showed that net nitrification rates in the upper mineral soil (0–10 cm) are still considerable and almost as high as gross nitrification. Even in the mineral soil layer from 10 to 40 cm, nitrification takes place (Matejek et al. 2010).

4.2 Tree roots

Horizontal and vertical distribution of fine and medium roots is well in accordance with other studies conducted at

this site (Kreutzer et al. 1991). The significant positive correlation with fine root biomass in the upper mineral soil and the inclusion of this parameter into the linear regression models suggests that roots in this depth take up water but not, or to a relative small extent NO_3^- , leading to an increase in $\text{NO}_3^-_{\text{seepage}}$. This is supported by a significant negative correlation between fine root biomass and water in sampling bottles (data not presented). Previous investigations from Gessler et al. (1998) showed that at the Höglwald NH_4^+ is taken up by tree roots in the organic layer and the uppermost mineral soil layer but not NO_3^- . The explanation that fine roots at our site grow into areas where N mineralization and nitrification is high was dismissed because there was no significant correlation between roots and N turnover parameters. At the Höglwald site, N supply with throughfall is around $30 \text{ kg ha}^{-1} \text{ year}^{-1}$, and most of it is in the form of NH_4^+ , which is preferred over nitrate by spruce trees (Gessler et al. 1998). Therefore, we assume that there is no need for trees to develop a specific fine root strategy to acquire N. In contrast, there was a significant negative correlation between $\text{NO}_3^-_{\text{seepage}}$ and fine roots in 30–40 cm depth, suggesting uptake of NO_3^- in this range. Marschner et al. (1991) reported significantly higher uptake of NO_3^- when NH_4^+ was absent in nutrient solution, which is the case at these depths at the Höglwald site. Within this study, we did not detect NH_4^+ in suction cup solution in 40 cm depth of the mineral soil, while in former investigations, NH_4^+ was still present in soil solution to a depth of 20–30 cm. These results suggest that fine roots in the upper mineral layer (0–30 cm) are responsible for an enrichment of NO_3^- in soil solution by uptake of water and NH_4^+ , while in deeper mineral soil layers (30–40 cm), fine roots reduce nitrate concentration by NO_3^- uptake. This is also supported by seepage water flux calculations at the Höglwald site, where Kreutzer et al. (1998) found lower NO_3^- fluxes in 40 cm compared to 20 cm depth

4.3 Stand parameters

Although there was no significant correlation between basal area (“stand density”) around the suction cup and $\text{NO}_3^-_{\text{seepage}}$ total basal area in a diameter of 4 m around the suction cups was included as a significant variable in the linear regression models at most of the sampling dates with decreasing explanatory power at the end of the vegetation period. A high stand density caused lower concentrations of $\text{NO}_3^-_{\text{seepage}}$. Kohlpaintner et al. (2009) interpreted a high stand density with high N input but also high N uptake by tree roots. Additionally, the cooler and possibly drier microclimate at dense subplots is not favorable for N mineralization, leading to lower nitrate production.

4.4 Ground vegetation

Coverage of *O. acetosella* (L.) showed the second highest correlation with $\text{NO}_3^-_{\text{seepage}}$. As the biomass production of oxalis at our site is low, it is unlikely that it has a major influence on N cycling (Rodenkirchen 1995). High coverage of this plant may rather be seen as an indicator than a cause for high $\text{NO}_3^-_{\text{seepage}}$ (Kohlpaintner et al. 2009). Therefore, coverage of oxalis was not a significant and relevant parameter in most of the linear regression models. Highly significant positive correlations with N turnover parameters suggest that a high coverage of this plant is a consequence of enhanced available inorganic N (especially NO_3^-) and calcium, which is favorable for its growth (Rodenkirchen 1998). Coverage of mosses was significantly negative correlated with $\text{NO}_3^-_{\text{seepage}}$, which was also detected by Kohlpaintner et al. (2009). High moss cover may indicate relatively cool and shady conditions, which may lower mineralization and nitrification processes. Coverage by vascular plants (without oxalis) showed no significant correlation with $\text{NO}_3^-_{\text{seepage}}$, but there was a trend towards a negative relationship between these two parameters. Several studies also reported lower $\text{NO}_3^-_{\text{seepage}}$ at sampling places with high vegetation coverage (Kohlpaintner et al. 2009; Weis et al. 2001; Huber et al. 2004). However, coverage neither by mosses nor by vascular plants was considered in the linear regression models.

4.5 Chemical soil parameters

Soil pH measured in H_2O in the subsoil showed significant negative correlation with $\text{NO}_3^-_{\text{seepage}}$. On a regional scale, Dise et al. (1998) and Kristensen et al. (2004) also found significant negative relationship between dissolved inorganic nitrogen and pH in the B-horizon of European forests. They concluded that the meaning of this relation is unclear as the lower pH values at sites with high N leaching may be caused due to proton production during the excess nitrification and may therefore be an effect of the long term high N deposition. In our case, we assume that the lower pH values at high $\text{NO}_3^-_{\text{seepage}}$ are caused by the actual high nitrification rates in the organic layer at these sampling places. Nevertheless, pH in subsoil seems to be one of the few parameters, which is correlated with $\text{NO}_3^-_{\text{seepage}}$ on the regional scale as well as at the small scale.

However, none of the chemical soil parameters was a significant explaining variable in the linear regression models. Even C/N, which is an important parameter concerning NO_3^- leaching at the regional scale (Dise et al. 2009; van der Salm et al. 2007), was not included into linear regression models.

4.6 Physical soil parameters

The measured physical soil parameters showed no significant correlation with NO_3^- and were not considered in the linear regression models. At our homogeneous forest site, the variability of these parameters (CV mostly below 20 %) may not be high enough to cause measurable influence on the N transformation processes.

4.7 Throughfall

It was surprising that N input via throughfall showed no correlation with NO_3^- , although at the large scale, N input is a very significant predictor for nitrate leaching (Gundersen et al. 1998; Dise et al. 1998; Kristensen et al. 2004; Borken and Matzner 2004). Manderscheid and Matzner (1995a) did not find correlations between N input and NO_3^- at the stand level either. High N input is a prerequisite for N saturation and the associated high spatial variability of NO_3^- but shows not enough small-scale variability at our site (CV about 25 %) to significantly explain variability of NO_3^- leaching.

5 Conclusions

Biological parameters like net N mineralization, root distribution, and stand density explained most of the variability in NO_3^- . While the rate of net N mineralization seems to be decisive for NO_3^- production, roots mediate the NO_3^- in two ways. While the fine roots in the upper mineral soil are responsible for enhanced NO_3^- concentrations due to intensive water uptake, medium-sized roots and fine roots in deeper soil layers take up NO_3^- and cause dilution. High stand density has a negative influence on NO_3^- and causes lower NO_3^- .

In contrast, neither of the measured chemical and physical soil parameters nor the depth of the organic layer and N input at the sampling places were able to give sound explanations concerning NO_3^- in the linear regression models. All of these variables were relatively uniform at the study site, which is shown by CV mostly below 20 %, which is considerably lower than the CV for NO_3^- and most of the measured biological parameters. This suggests that at our homogeneous site biological factors and processes are the main drivers of variation in N leaching, while abiotic parameters play only a minor role, which cannot be resolved in our models. This may be different at sites where these abiotic soil parameters are more heterogeneous like in mountainous regions.

Important parameters that explain variation of nitrate leaching on the regional scale like N input by throughfall

and C/N ratio can obviously not explain leaching at the small scale in homogeneous N saturated stands.

Some of the explaining variables were measured only once and at different times or time steps during the vegetation period. Now, as we know which parameters are responsible for the variability of NO_3^- , repeated and synchronous measurements of these variables would yield better information about the temporal variability and may even improve the explaining power of the models. As the measurement of net N mineralization is not very complicated, this parameter should be used to further explore NO_3^- leaching in N saturated forests.

Furthermore, the turnover of the root biomass should be considered for better temporal resolution. In addition, the high spatial variability of the NO_3^- and its explaining variables requires a sampling design with the ability to detect high spatial resolution in order to gain representative information.

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