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Estimation of net nitrification in mountain soils

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Abstract
The method of measuring nitrification rate in grassland and acid forest soils of the mountain ecosystems, which would facilitate comparison of different soils, was evaluated. It is recommended to sample soil for analysing in early spring, when the moisture is not limiting microbial activity, and to store homogenised samples for 3–4 weeks at 4°C before the measurements to relieve the effect of soil sampling. Then the soil should be incubated without addition of N substrate at temperature below 20° C to avoid inhibition of nitrification rate. The measurement of net nitrification in the soil between the 1st and 3rd week of incubation appears to be the most appropriate as the possible effect of both soil handling at the beginning of incubation and artefacts caused by a too long incubation period are excluded.

Key words: catchment, method

Introducion

It has been generally accepted that nitrification might be of importance in natural grassland (Davidson & al. 1990). Recent studies on nutrient cycling in small montane catchments, have however indicated that nitrification can be an important component of the internal N cycle also in coniferous forests (e.g. Guldersen & Rasmussen 1990, Killham 1990, Davidson & al. 1992). Nevertheless, net nitrification rates, defined as the accumulation of NO_3-N during aerobic soil incubation in the absence of plant root uptake, are often found to be very low or negligible in acidic soils. This is partly due to methodological difficulties connected with a significant lag period before the onset of measurable NO_3^- production, often following disturbance of the soil environment caused by the soil sampling.

Basically, there are two laboratory techniques available for the estimation of nitrification rates in soils. Short-term nitrification assay, which is based on the evaluation of NO_3-N increase in soil slurry during several-hour incubation, has been successfully applied to agriculture soils and soils with pH>5 (Alef & Nannipieri 1995). The nitrification rate is obviously too low in most acidic soils to produce detectable amount of nitrates in a short period. Long-term nitrification assay, when the soil is kept at optimum moisture, temperature and substrate (NH_4^+) concentration for at least several weeks or even several months, was found to be more suitable from this point of view (Alef & Nannipieri 1995). During prolonged incubation the lag phase of nitrification activity is easily overcome and relatively low rates of nitrate accumulation can be measured. According to our experience, the main disadvantage of any long-term incubation assay is that change in the composition of the soil microbial community prob-

ably occurs during the incubation. It is thus not easy to extrapolate the long-term laboratory incubation data to field conditions.

The goal of the present study was to evaluate a technique of estimating net nitrification rate that could be used for both, the grassland and acid forest mountain soils and which would allow measurement of net nitrification in the course of few weeks. The use of the same method in all principal mountain terrestrial ecosystems facilitates studies of nutrient cycling in catchments.

Materials and Methods

Soil samples were collected from the mountain grassland at the site Huťská Hora in the Bohemian Forest (Zhůří, abbreviated here ZH), from an alpine meadow in the Tatra Mts. in Slovakia (Wahlenbergovo pleso lake; WA) and from spruce forest in the Bohemian Forest (Čertovo Lake, CT) in early spring of 2000. Five to 9 subsamples were taken from the 0–10 cm soil layer at the ZH grassland, from A horizon in the WA meadow and from A, AB and C soil horizons in the CT forest. Representative samples used for analyses were prepared by mixing aliquot parts of soil from the same horizons. Soil samples were sieved (5 mm), homogenized and stored in polyethylene bags at 4°C until they were processed (after 3–4 weeks). Basic soil characteristics are given in Table 1, for more detailed characteristics of ZH and CT soils see Kvítek & al. (2001) and Kopáček & al. (2000).

The method used for measurement of nitrification rate represents a combination of techniques by Ste-Marie & Paré (1999) and Zhu & Carreiro (1999). Moist soil (20 g of mineral soil, 10 g of organic soil, 6 replicates) was placed into a 100 ml Ehrlenmayer flask and soil water content was adjusted to 50% maximum water holding capacity (the soil weight was checked weekly and distilled water was added if necessary to maintain the original soil moisture). Three replicates were used for determination of initial mineral nitrogen concentration (NH_4^+, NO_3^-) in the soil. The others were sealed with perforated parafilm and kept at 20°C in darkness for up to five weeks. Mineral N concentrations were measured destructively in 3 replicates at the end of incubation. When the effect of incubation period was tested, the soil samples were incubated at 20°C for 1, 2, 3, 4 and 5 weeks, respectively. When the effect of temperature was tested, the soil was incubated at 15, 20, 25 and 30°C for 2 and 4 weeks, respectively. When the effect of substrate level was tested, $(NH_4)_2SO_4$ solution was dropped on the soil surface to the final concentration of 200 μg $N.g^{-1}$ at the beginning of incubation and soil was incubated at 20°C for 2 and 4 weeks, respectively. At the beginning of incubation and on each sampling date, mineral N (NH_4^+ and NO_3^-) was extracted from each subsample by adding 2 M KCl (1 g dry mass equivalent in 2 ml KCl) and shaking for 1 hour. The extract was then centrifuged (10000 g, 10 min), the supernatant was filtered through glass fibre filter (0.45 μm) and immediately analysed for NH_4^+. The filtrate for NO_3^- detection was kept

Table 1. – Soil moisture, pH, C content (C_{tot}), C/N ratio, mineral N content (NH_4^+ and NO_3^-), microbial biomass (C_{mic}) and mineralization rate (C_{min}) in the studied soils.

soil	moisture (% H_2O)	pH_{H_2O}	C_{tot} $g.m^{-2}$	C/N	NH_4-N $g.m^{-2}$	NO_3-N $g.m^{-2}$	C_{mic} $g.m^{-2}$	C_{min} $g.m^{-2}.d^{-1}$
grassland – ZH		3.8			0.17	1.61	84.3	1.83
grassland – WA		4.5	2348	13.3	0.38	0.67	43.5	1.24
forest – CT, A ho	62	3.6	6129	20.2	0.047	0.115	29.2	2.26
AB ho	41	3.9	3907	18.6	0.076	0.36	21.9	1.51
C ho	21	4.5	2602	28.4	0.055	0.044	6.2	0.82

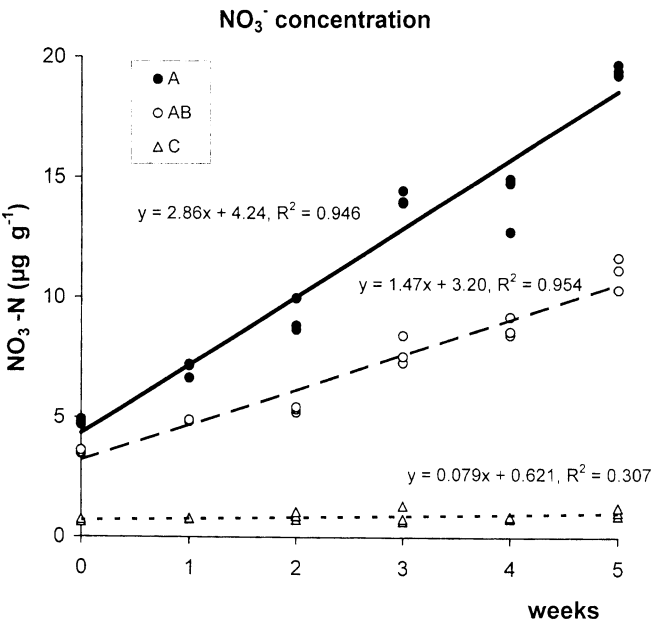

Fig. 1. – Change of NO_3^- concentration in different horizons of CT soil during 5 week incubation. The dependence of NO_3^- concentration on time was fitted using linear regression.

frozen (–10°C) until being analysed. NH_4^+ and NO_3^- were analysed by flow injection analyser (Tecator FIAStar 5020). Daily net nitrification rate was calculated as the difference between final and initial NO_3^- concentrations divided by the number of days. Net ammonification rate was calculated in the same manner but using mineral NH_4^+ concentrations.

Results

Concentration of NO_3^- increased linearly during 4 (5) weeks of incubation (Figs. 1 and 2) in all soils under study. The increase in NO_3^- concentration was significant except for the mineral C horizon of acid forest soil (Fig. 1).

The response of net nitrification and net ammonification rates to temperature were measured after 2 and 4 weeks of incubation in the meadow (WA) and forest (CT) soils. The length of incubation period and the soil properties affected the temperature response (Fig. 3). Generally, net nitrification rate was lower in CT than in WA soil and it increased between 15 and 20°C in all cases. It decreased at temperature above 25°C. The response of net ammonifica-

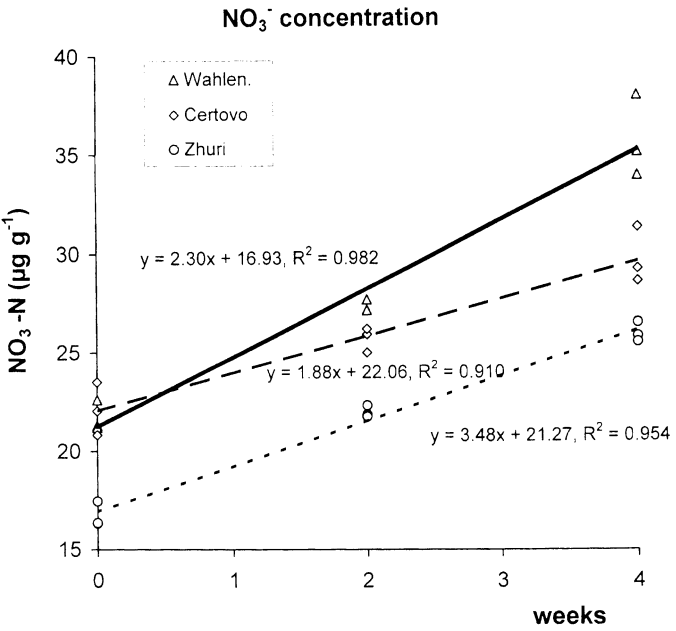

Fig. 2. – Change of NO_3^- concentration in WA, CT and ZH soils during 4 week incubation. The dependence of NO_3^- concentration on time was fitted using linear regression.

tion rate (ammonium production) followed a different pattern. It increased more or less gradually with increasing temperature. As a result, net ammonification significantly surpassed net nitrification when temperature exceeded 25°C.

Table 2. – Net nitrification rate (ng $N.g^{-1}.d^{-1}$) in the soils incubated at 20°C without and with addition of N substrate (0 and 200 µg $N.g^{-1}$ as $(NH_4)_2SO_4$, respectively). Mean values and standard deviations from 4 replicates are given.

soil	2 weeks of incubation		4 weeks of incubation	
	0	200 µg $N.g^{-1}$	0	200 µg $N.g^{-1}$
ZH	397.1±3.3	486.4±33.7	359.3±19.5	441.8±41.8
WA	399.3±19.9	–91.4±3.75	579.3±42.6	–1.75±0.06
CT (A hor)	254.2±6.23	–2.14±0.05	268.9±27.6	–0.064±0.002

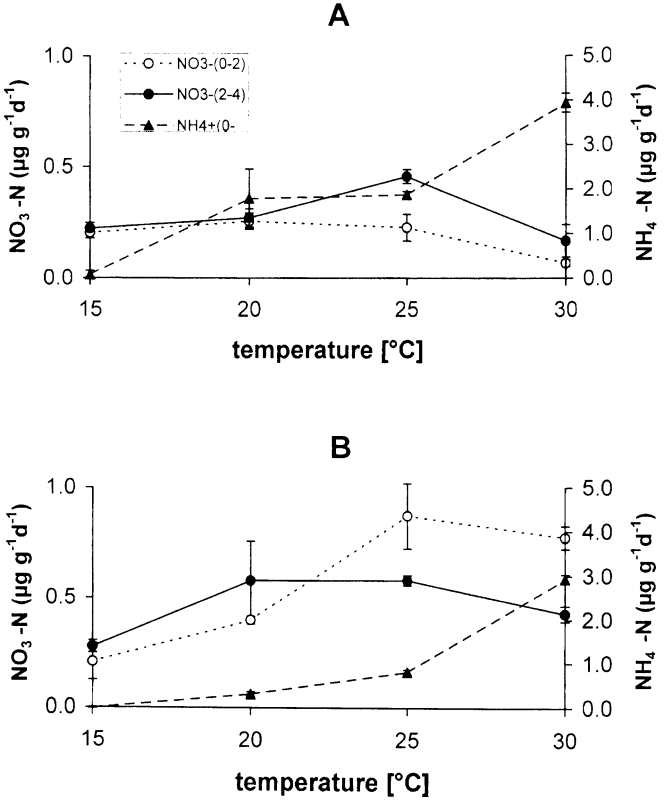

Fig. 3. – Dependence of net ammonification (NH_4^+) and net nitrification rates after 2 weeks (NO_3^- (0–2)) and after 4 weeks (NO_3^- (2–4)) of incubation on temperature: (A) in CT soil and (B) in WA soil. Mean values and standard deviations from 4 replicates are given.

The effect of N substrate amendment ($(NH_4)_2SO_4$) was tested in all soils. The substrate addition increased net nitrification only in the ZH soil. The addition of N substrate to WA a CT soils resulted in a strong inhibition of net nitrification rate (Table 2).

Discussion

The laboratory incubation methods might bring skewed assessment of net nitrification as the measurements are carried out in disturbed soil that is separated from roots and incubated under optimum conditions which need not to be typical of the field conditions. However, the laboratory methods are appropriate for comparisons of different soils from various ecosystems. Paré & Bergeton (1996) even found good agreement between laboratory incubation methods and *in situ* measurements.

Our results indicate that the incubation period of 2 to 4 weeks is sufficient to determine nitrification in both, the meadow and acid forest montane soil and that an increase in NO_3^- content in the soil remains constant over the incubation period. We did not detect any lag period which is often observed at the beginning of incubation, namely after remoistening of dry soil (Alef & Nannipieri 1995). A plausible explanation that the procedure we used restored the effect of disturbation and remoistening, as the soil was sampled in early spring when the field moisture was not a limiting factor and it was stored for 3 weeks before analyses.

It also appears that net nitrification is not controlled by low ammonium availability during incubation, which coincides with the observations by Ste-Marie & Paré (1999). We even observed inhibition of net nitrification by addition of N substrate. The inhibition may have been caused (i) by sulphate form of N substrate which could have induced lowering of pH or (ii) too high concentration of ammonium nitrogen in the soil, where microbes are adapted to low ammonium concentration.

The incubation temperature usually used for measuring net nitrification rate ranges between 20°C and 25°C and the optimum temperature for nitrification is referred to be 25°C and more (Focht & Vestraete 1977). The temperature of 20°C was chosen in this study, as we expected a sort of adaptation of soil nitrifying microorganisms to naturally low temperatures prevailing during the year in the soils under study. Malhi & McGill (1982) reported the optimum of 20°C in soils from a site with mean annual temperature of 2.5° C. Thamdrup & Fleischer (1998) observed notable adaptation of nitrification in Arctic sediments to low temperature. It is obvious that microbes from cold conditions are adapted to lower temperatures: this should be taken into account when measuring soil processes. Thus we recommend the maximum temperature of 20°C for measuring net nitrification rate in mountain soils.

Conclusion

The measurement of net nitrification in mountain soils without addition of any nitrogen is possible after an incubation period of up to 4 weeks. The increase in nitrate concentration in the soil during the incubation is above the detection limit of available analytical techniques. The method is suitable for comparisons of soils from different ecosystems and/or treatments. We recommend to analyse soils sampled in early spring, when the moisture is not limiting microbial activity, and to store homogenised samples for 3–4 weeks at 4°C before the measurements to relieve the effect of soil sampling. Measurement of net nitrification between the 1st and 3rd week of incubation appears to be the most appropriate as the possible effect of both soil handling at the beginning of incubation and artefacts caused by an excessively long incubation period are excluded.

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