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Short communication

Cold storage and laboratory incubation of intact soil cores do not reflect in-situ nitrogen cycling rates of tropical forest soils

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ABSTRACT

Measurements of N transformation rates in tropical forest soils are commonly conducted in the laboratory from disturbed or intact soil cores. On four sites with Andisol soils under old-growth forests of Panama and Ecuador, we compared N transformation rates measured from laboratory incubation (at soil temperatures of the sites) of intact soil cores after a period of cold storage (at 5 °C) with measurements conducted in situ. Laboratory measurements from stored soil cores showed lower gross N mineralization and NH_4^+ consumption rates and higher gross nitrification and NO_3^- immobilization rates than the in-situ measurements. We conclude that cold storage and laboratory incubation change the soils to such an extent that N cycling rates do not reflect field conditions. The only reliable way to measure N transformation rates of tropical forest soils is in-situ incubation and mineral N extraction in the field.

Nitrogen (N) status of tropical forests has been shown to be the key to analyze how forest ecosystems will react to predicted changes in N deposition in the tropics (Vitousek and Farrington, 1997; Tanner et al., 1998). Soil N status has been commonly assessed by measurements of mineral N concentrations and N transformation rates. These are preferably done on fresh soil samples. However, often this is done in the laboratory which involves cold storage and pre-incubation. Cold storage of soil samples at 2–5 °C is recommended for temperate soils (Wollum, 1982; Hart et al., 1994) and is widely used when soils cannot be processed directly after sampling. Refrigeration is supposed to decelerate microbial growth and to decrease disturbance effects associated with sampling. While microbial populations in temperate soils are adapted to a large range of temperature, including values below the freezing point, microorganisms in tropical soils are accustomed to relatively high temperatures with small fluctuations. Thus, cooling to temperatures of 2–5 °C is abnormal for microbial biomass in these soils and repression or stimulation of microbial processes, such as mineralization and nitrification, may occur during cold storage or rewarming of samples after storage. Hence, cold storage and subsequent laboratory incubation may lead to significant alterations in microbial activities, resulting in modified nutrient availabilities. Moreover, the delay between sampling and measurement of N transformation rates in tropical soils, which commonly have high N cycling rates, may falsify results more seriously than in temperate soils which usually have lower N cycling rates.

Nevertheless, most previous studies investigating N dynamics in tropical forest soils involve cold storage of samples between collection and laboratory measurement (e.g. Neill et al., 1999; Hall and Matson, 2003; Corre et al., 2006; Sotta et al., 2008). The extractable NH_4^+:NO_3^- ratios have been used to indicate N status in ecosystems, with a declining ratio when N availability increases (Vitousek et al., 1982; Davidson et al., 2000). This statement may not be applicable for measurements conducted from stored-laboratory incubated soils, if storage and laboratory incubation lead to a shift in NH_4^+:NO_3^- ratios due to changes in mineral N production rates and/or microbial biomass. Our objective was to investigate how cold storage and laboratory incubation of tropical forest soils change N transformation rates compared to in-situ measurement.

The study was conducted in Andisols under old-growth forests in Panama and north-western Ecuador. In Panama (Fortuna site, 1200 masl), the soil is classified as a Hapludand. We sampled at 8 sampling points (considered as replicates) with a minimum distance of 80 m. At each point, 12 intact soil cores were taken within a 0.6-m^2 area using stainless steel cores of 5-cm height and 8-cm diameter. Soil cores were taken after removing fresh and partially decomposed litter, and hence the soil samples encompassed the horizon below this loose litter down to 5-cm depth. Six of the cores were incubated in situ and extracted for mineral N right in the field by bringing prepared bottles of 150 ml 0.5 M K_2SO_4 solution to

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which soil samples (approximate solution to dry mass soil ratio of 5) were added (hereafter referred to as in-situ measurement). The soil–K_2SO_4 bottles were brought in a cooler from the field to the laboratory, shaken for 1 h, filtered, and the extracts were frozen immediately. The other 6 cores were put in a cooler in the field and brought to the laboratory where they were refrigerated at 5 °C for 2 days followed by 3-day acclimatization, incubation and extraction in the laboratory at 20 °C (soil temperature of the site) (referred to as stored-laboratory measurement). The 3-day acclimatization was undertaken to recondition the microbial activity and to avoid artificially low N cycling rates possibly due to the cooling. In Ecuador, the soils are classified as Fulvudands for Pitzara (300 masl) and La Bilsa (630 masl), and Hapludand for Mindo (1500 masl). At each site, 5 sampling points (or replicates) spaced between 25–50 m were randomly selected. We sampled 6 cores from each point as described above. Soils were sampled in 2005 and 2006 during the rainy season. The 2005 samples were cooled immediately after sampling and were stored at 5 °C for 30 days followed by 3-day acclimatization, incubation and extraction in the laboratory at temperatures similar to the soil conditions of the sites (23 °C for Pitzara, 22 °C for La Bilsa, and 18 °C for Mindo). The 2006 samples were incubated and extracted in situ, as described for in-situ measurement of the Panama site. Time between field extraction and filtration ranged from 3 (Pitzara) to 7 h (Fortuna and Mindo), depending on the distance of the sites to the laboratory.

For each method (in-situ and stored-laboratory measurements), 4 of the 6 soil cores were used for the determination of gross rates of N cycling using the ^{15}N pool dilution techniques (Davidson et al., 1991; Hart et al., 1994). Two cores were injected with 130 μg N–$(NH_4)_2SO_4$ (96% ^{15}N) contained in 5 ml solution (for gross N mineralization and NH_4^+ consumption), and each of the other two cores with 130 μg N–KNO_3 (99% ^{15}N) in 5 ml (for gross nitrification and NO_3^- immobilization). These are equivalent to a rate of 1 μg N g^{-1}. One core of each labeled pair was extracted with 0.5 M K_2SO_4 after 10 min and the other core was incubated for 1 day and then extracted. Microbial immobilization of NO_3^- was determined by 5-day $CHCl_3$-fumigation of the 1-day incubated, $^{15}NO_3^-$-injected cores. This was only measured from the Ecuador sites because immediate fumigation was not possible for the Panama site. The two remaining cores were used for measurements of initial mineral N concentrations and net N transformation rates with 7-day incubation period. Soil extracts remained frozen during transport by air to the University of Goettingen (Germany), where ^{15}N diffusion and mineral N analyses were conducted. For ^{15}N diffusion, 50 ml of extract was placed in a 150 ml glass bottle. NH_4^+ was diffused from the $^{15}NH_4^+$-labeled cores by adding MgO to the extracts, placing immediately the acid trap (2 discs of 7-mm diameter glass fiber filter paper acidified with 20 μl of 2.5 M $KHSO_4$ and encased in 5-cm wide Teflon tape) on the mouth of the bottle, and fastening the lid tightly. Diffusion proceeded for 6 days. NO_3^- was diffused from the $^{15}NO_3^-$-labeled cores by first adding MgO to the extracts and leaving the bottles open for 6 days to get rid of NH_4^+, followed by 6 days of diffusion after adding Devarda's alloy to convert NO_3^- to NH_4^+ and eventually to NH_3 (Corre et al., 2006; Sotta et al., 2008). For the 1-day incubated, $^{15}NO_3^-$-injected cores, ^{15}N enrichment in the microbial biomass was determined by persulfate digestion of the extracts from fumigated and unfumigated soils, and diffusion was carried out by adding 2 ml of 10 M NaOH and Devarda's alloy to convert persulfate-N (in NO_3^- form) to NH_3 (Corre et al., 2007). Gross rates of N mineralization, NH_4^+ consumption and nitrification and NO_3^- immobilization rates were calculated using the equations provided by Davidson et al. (1991). Statistical differences between measurement methods for each site were assessed using the Mann–Whitney U Test at $P \leq 0.05$ and correlation analysis using Spearman's rank correlation test, as assumptions for normal distribution and equality of variance were not met.

Our results showed that NH_4^+ concentrations were slightly higher in in-situ than in stored-laboratory measurement except at one site (Fig. 1A), but NO_3^- concentrations strongly increased in stored-laboratory measurement (Fig. 1B). Gross N mineralization rates were higher in in-situ than in stored-laboratory measurement for all sites, although these differences were statistically significant only at two Ecuadorian sites (Fig. 1C). These two Ecuadorian sites also showed significantly higher net N mineralization rates in in-situ than in stored-laboratory measurement. At all sites, gross nitrification (Fig. 1D) and net nitrification rates were much lower in in-situ than in stored-laboratory measurement. Only 1–12% of the mineralized N was nitrified in situ while 37–100% of the mineralized N was transformed to NO_3^- in stored-laboratory measurement. The increased gross nitrification rates in stored-laboratory measurement were paralleled with decreased NH_4^+ assimilation rates (NH_4^+ consumption–gross nitrification). NH_4^+ consumption rates decreased in stored-laboratory measurement (Fig. 1E) whereas the converse was true for microbial immobilization of NO_3^- (Fig. 1F) compared to in-situ measurement. Soil moisture contents did not differ between in-situ and stored-laboratory measurement in all but one site (Mindo). For the Mindo site, soil moisture was higher in in-situ than in stored-laboratory measurement and water-filled pore space was correlated with gross nitrification rates ($r = -0.64$; $P = 0.05$), indicating that for this site the increased gross nitrification rates in stored-laboratory measurement could be partly due to the change in soil aeration status.

The decrease in N mineralization in stored-laboratory measurement was probably due to decrease in easily mineralizable organic N with storage, especially in the long-term stored soil cores of the Ecuador sites. Reduced availability of organic matter with storage was reflected in the significant decrease of microbial biomass C in all Ecuador sites. The microbial C in stored-laboratory measurement was only 32–64% of those in in-situ measurement. With the absence of plant uptake during sample storage and laboratory incubation, the nitrifiers were probably able to compete more for available NH_4^+, resulting in increased nitrification rates. This was observed even in the shortly stored soil cores of the Panama site. In a study on effects of low temperatures on N transformation rates, Cookson et al. (2002) measured gross nitrification at 2 and 5 °C. The possible sustained nitrification activity during cold storage combined with favoured nitrification activity under laboratory incubation could have resulted in the dominance of NO_3^- over NH_4^+, with the lowest NH_4^+:NO_3^- ratio in the site (La Bilsa) with highest gross nitrification rates. The increased NO_3^- availability in stored-laboratory incubated soil cores consequently led to the enhanced uptake of NO_3^- by microbial biomass.

Verchot (1999) examined nitrification potential of Brazilian Oxisols under primary forests after 5-day cold storage of mixed soils, and although nitrification potential decreased in most of the sites, he observed a very high rate of NO_3^- production from stored soils of one primary forest site. To our knowledge, our present study is the first to report how storage and subsequent laboratory incubation of tropical soils under old-growth forests affect N transformation rates. Neill et al. (1999) reported 57–70% gross nitrification of gross N mineralization from mixed soils stored cold up to three weeks followed by laboratory incubation. Other studies on soil N cycling in old-growth tropical forests (e.g. Hall and Matson, 2003; Silver et al., 2005; Sotta et al., 2008) included storage of soils (mixed or intact cores) with subsequent laboratory incubation or at least transport of mixed soils to the laboratory from in-situ incubated cores prior to extraction, and all these studies showed higher ratios of gross nitrification to gross N mineralization rates than our in-situ measurement of the present sites. Although these data are from different soil types, climatic conditions, and extraction methods, relatively high ratios of gross nitrification to gross N mineralization rates in these studies support our present results.