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Stimulated activity of the soil nitrifying community accelerates community adaptation to Zn stress

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ABSTRACT

Field data have shown that soil nitrifying communities gradually adapt to zinc (Zn) after a single contamination event with reported adaptation times exceeding 1 year. It was hypothesized that this relatively slow adaptation relates to the restricted microbial diversity and low growth rate of the soil nitrifying community. This hypothesis was tested experimentally by recording adaptation rates under varying nitrification activities (assumed to affect growth rates) and by monitoring shifts in community composition. Soils were spiked at various Zn concentrations (0–4000 mg Zn kg^{-1}) and two NH_4^+–N doses (N1, N2) were applied to stimulate growth. A control series receiving no extra NH_4^+–N was also included. Soils were incubated in pots under field conditions with free drainage. The pore water Zn concentration at which nitrification was halved (EC50, mg Zn l^{-1}) did not change significantly during 12 months in the control series (without NH_4^+–N applications), although nitrification recovered after 12 months at the highest Zn dose only. The EC50 after 12 months incubation increased by more than a factor 10 with increasing NH_4^+–N dose ($p < 0.05$) illustrating that increased activity accelerates adaptation to Zn. Zinc tolerance tests confirmed the role of Zn exposure, time and NH_4^+–N dose on adaptation. Zinc tolerance development was ascribed to the AOB community since the AOB/AOA ratio (AOB = ammonia oxidizing bacteria; AOA = ammonia oxidizing archaea) increased from 0.4 in the control to 1.4 in the most tolerant community. Moreover, the AOB *amo*A DGGE profile changed during Zn adaptation whereas the AOA *amo*A DGGE profile remained unaffected. These data confirm the slow but pronounced adaptation of nitrifiers to Zn contamination. We showed that adaptation to Zn was accelerated at higher activity and was associated with a shift in soil AOB community that gradually dominated the nitrifying community.

1. Introduction

Microorganisms play an important role in nutrient cycling in soil and should be protected to maintain soil quality. Contrasting views exist if and how effects of soil contaminants on microorganisms should be considered in regulation of soil trace metals. An argument against their inclusion is that microorganisms have sufficient capacity to adapt to changing environments due to functional redundancy within the microbial community (Kapustka, 1999). Clearly, the mechanisms and ecological relevance of microbial adaptation requires more information.

Adaptation of a microbial community is well reported for soil contaminated with toxic trace metals such as zinc (Zn), copper (Cu) and cadmium (Cd), and the occurrence of trace metal tolerance has frequently been shown for microbial communities exposed to trace metals in the long term (e.g. Diaz-Raviña et al., 1994; Almås et al., 2004; Davis et al., 2004). The development of trace metal tolerance of microbial communities is attributed to the selection and proliferation of metal tolerant or genetically changed microorganisms (Diaz-Raviña and Bååth, 1996; Giller et al., 1998). As a consequence, the microbial community can change structurally upon trace metal adaptation (Kelly et al., 1999; Mertens et al., 2006). Studies on the microbial adaptation rate in soil revealed significantly increased tolerance after 2 days–4 months exposure depending on the trace metal concentration and the studied microbial community (Diaz-Raviña and Bååth, 1996).

The nitrification process is one of the most sensitive microbial assays to indicate trace metal toxicity (Broos et al., 2005). Field and laboratory data have shown that the soil nitrifying community does not adapt to Zn within 6 months (Mertens et al., unpublished data), but does after 1–2 years at soil total concentrations of 780 mg Zn kg^{-1} (Rusk et al., 2004) and 1850 mg Zn kg^{-1}

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(Mertens et al., 2009). As a consequence, this process is highly suited to study responses to trace metal additions. The soil ammonia oxidation process, the first and rate limiting step in the nitrification process, has long time been thought to be performed by a select group of β-Proteobacteria (Kowalchuk and Stephen, 2001). However, archaeal ammonia oxidation has recently been shown in marine systems (Könneke et al., 2005), and archaeal *amo*A genes resembling its bacterial counterpart have shown to be abundantly present in soil (Treusch et al., 2005; Leininger et al., 2006). Nevertheless, the actual contribution of ammonia oxidizing archaea (AOA) to the soil nitrification process is still to be determined (Prosser and Nicol, 2008).

The aim of this study was to monitor the Zn adaptation rate and ammonia oxidizing bacteria (AOB) and archaea (AOA) community composition in Zn spiked soils at varied microbial activities. We hypothesized that ammonium applications, stimulating the activity and, hence, the growth rate of the nitrifying community (Taylor and Bottomley, 2006), accelerate the induction of Zn tolerance within the nitrifying community. Ammonium fertilization has previously been shown to increase the abundance of the ammonia oxidizing bacteria and archaea with demonstrated shifts in community structure (Okano et al., 2004; Le Roux et al., 2008). However, long term NPK fertilization did not affect the sensitivity of the nitrifying community to Zn after 8 weeks exposure (Xia et al., 2007). Based on this, a 1-year experiment was set up with soils spiked at various Zn concentrations (0–4000 mg Zn kg^{-1}) in a factorial design with a control series (N0) and two NH_4^+–N doses (N1, N2). The rate of adaptation and extent of Zn tolerance in the three series was recorded and correlated with shifts in AOB and AOA community abundance and composition. Care was taken to correct for pH shifts among treatments by adding CaO since soil pH affects both the nitrification activity and the Zn bioavailability.

2. Materials and methods

2.1. Soil sampling

Uncontaminated top soil (0–20 cm) was sampled from grassland in Zeveren (Belgium) in June 2007. Uncontaminated top soil and zinc contaminated soil sampled near the electricity transmission pylon at this site was used before to show the occurrence of Zn tolerance of the AOB community due to long term exposure to elevated Zn concentrations (Mertens et al., 2006). Fresh soil was collected, sieved (<4 mm) immediately after sampling and stored in plastic barrels at 4 °C in the dark until use. Selected soil characteristics were measured as described previously (Smolders et al., 2004). The soil has a sandy loam texture with a cation exchange capacity of 21 $cmol_c$ kg^{-1}, 3.5% organic matter, soil pH (0.01 M $CaCl_2$) of 5.6, C/N ratio of 10 and a total Zn concentration of 60 mg kg^{-1}.

2.2. Experimental set up

In August 2007 the soil was artificially contaminated with increasing Zn doses by adding dissolved $ZnCl_2$ to final total zinc concentrations of 60 (control soil – no added Zn), 82, 100, 194, 734, 1303 and 4058 mg Zn kg^{-1} dry soil. Soil samples of this Zn series are marked as Zn0–Zn6 with increasing Zn concentration. Perforated plastic pots (volume 2 dm^3) filled with 1.5 kg soil were incubated in open air. The pots were installed in a container filled with sand to insulate soil and to allow free drainage. Soils were weeded periodically.

Two months after Zn addition, soil of each Zn treatment was homogenized, sampled and split into 3 subseries receiving different NH_4^+–N doses: a control series without NH_4Cl application (N0 series), the N1 series receiving 80 mg NH_4^+–N kg^{-1} dry soil (equivalent to about 150 kg N ha^{-1}) and the N2 series receiving 160 mg NH_4^+–N kg^{-1} dry soil (about 300 kg N ha^{-1}). Each of the 3N × 7Zn treatments was performed in duplicate. A second NH_4^+–N application with the same doses was conducted 8 months after Zn addition, i.e. 6 months after the first NH_4^+–N application.

Soil was sampled within one week after Zn addition ('T0') and after 2 months ('T1' – i.e. prior to the first ammonium addition), 6 months ('T2' – i.e. 4 months after the first NH_4^+–N application) and 12 months ('T3' – i.e. 4 months after the second NH_4^+–N application) exposure to Zn. Prior to sampling, the soil in the pots was homogenized. Soil pH dropped with increasing Zn doses due to specific Zn^{2+} sorption and with increasing NH_4^+–N applications due to the nitrification. Therefore, pH of the residual soil was adjusted to pH 5.5 ± 0.2 by mixing ground CaO powder into the soil (doses based on pH titration curves) at the start of the experiment (T0) and after each sampling (T1 and T2). At T3, soil pH was corrected one week prior to the bioassay by adding $Ca(OH)_2$. Treatments which did not receive CaO or $Ca(OH)_2$ were subjected to the same mixing procedure.

At each sampling, soil pH (0.01 M $CaCl_2$), total Zn (aqua regia digestion) and pore water Zn concentrations (double chamber method; Smolders et al., 2004) of all treatments were determined.

2.3. Potential nitrification rate (PNR)

The soil nitrification process was quantified by measuring the PNR as described (Smolders et al., 2001). Briefly, soil moisture content was corrected to 47% (pF = 2) and soil was preincubated for 7 days at 20 °C in darkness. Afterwards, NH_4Cl solution (15 mg ml^{-1}) was added to the soil to a final added concentration of 100 mg NH_4^+–N kg^{-1} dry soil. Subsamples were taken immediately after NH_4^+–N addition and after 3 days incubation at 20 °C. Potassium chloride 1 N was added to the samples (1:5 solid:liquid ratio), samples were shaken end-over-end for 2 h, centrifuged (3000 g, 15 min) and the NO_3^-–N concentration was measured colorimetrically (SA 40, Skalar, The Netherlands). The PNR was calculated by linear regression as the increase of the soil nitrate concentration in dry soil over time (mg NO_3^-–N kg^{-1} d^{-1}). The Zn doses at which the PNR was 50% reduced compared to the control treatment (Zn0) (EC50 values) were expressed as Zn concentrations in pore water, mimicking the bioavailable Zn concentration (Mertens et al., 2007). The recovery of the nitrification process over time, quantified by increasing EC50 values, is therefore indicative of adaptation of the nitrifying community to Zn.

2.4. Zn tolerance testing

Zn tolerance was tested using the spike-on-spike test described by Mertens et al. (2006). Briefly, $ZnCl_2$ (50 mg ml^{-1}) was added to soil samples suspended in $CaCl_2$ 0.01 M (1:10 solid:liquid ratio) to final concentrations of 0, 30, 100, 300 and 1000 mg added Zn l^{-1}. Ammonium chloride solution (15 mg ml^{-1}) was added to a final concentration of 10 mg NH_4^+–N l^{-1}. The pH in suspension was adjusted immediately after N addition and daily during the 3-day test to pH 5.5 ± 0.1 using 0.1 M NaOH or HCl if required. Nitrate concentrations were measured as described above at the start of the experiment and after 3 days, and the PNR was calculated. The PNR in suspension was related to Zn concentrations in the $CaCl_2$ soil extract. Soluble Zn in the extract was measured in the supernatant after centrifugation (3000 g, 15 min) with ICP-OES (Perkin Elmer Optima 3300 DV, Norwalk, CT, USA). Zn tolerance was expressed as the soluble Zn concentration at which the PNR was 50% reduced compared to the control treatment. The Zn tolerance was tested in