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Chloride concentration affects soil microbial community

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Abstract

We studied the effect of increased inorganic chloride concentration on forest soil microflora in a laboratory experiment. Microbial DNA extracted from experimental soil samples was amplified with PCR using primer pairs specifically amplifying bacterial, eukaryotic and fungal DNA fragments. The resulting amplified DNA was further used for terminal restriction fragment length polymorphism (TRFLP) analysis. Our work revealed that chloride concentration affects the indigenous microbial community in experimental soil. This was documented on an unidentified microorganism whose DNA was detectable in soil high in chloride but was not found in soil with low chloride concentration. The presence of the organism responsive to increased chloride concentration was associated with the highest observed value of chlorination of humic acid, suggesting possible role of this organism in soil chlorine turnover. High chloride concentration in the soil tended to decrease the rate of degradation of trichloroacetic acid. The problems connected with measurement of chlorination rates in soil are discussed.

Keywords: Soil chloride; Terminal restriction fragments; Soil microorganisms; Organic matter chlorination; Trichloroacetic acid; Humic substances

1. Introduction

Inorganic chloride is a component of soil solution which is common both in coastal regions, where it is imported by aerosols formed by sea water (Meira et al., 2006), and in areas along salted roads, where sodium chloride is annualy applied to avoid road surface freezing, with consequent biological effects (e.g. Davidson, 1971; Bester et al., 2006).

The observations indicating decreased diversity of plant community under increased chloride impact (e.g. Lymbery et al., 2003) suggest that similar effects of increased chloride concentrations may occur, in analogy, also in underground soil biota. However, to our knowledge, no field data have been published on the effects of increased salinity on communities of soil microorganisms.

Specific response of different species of soil microorganisms to increased chloride concentration should result in changed composition of soil microbial community. Moreover, soil inorganic chloride enters a process of chlorination of soil organic matter (Öberg et al., 1996, 1997), mediated mainly by microorganisms and microbially produced exoenzymes (Asplund and Grimvall, 1991; Asplund et al., 1993).

Ecologically important may be the chlorination of humic substances (fulvic acid and humic acid) which was observed in vitro by Niedan et al. (2000) and Matucha et al. (2003a), in the presence of microbial chloroperoxidase and hydrogen peroxide. Humic substances are considered a resistant soil organic matter fraction with a long turnover time, whose recycling proceeds mainly by oxidative degradation.

The chlorination process enhances the cleavage of aromatic rings in these compounds (Matucha et al., 2007) producing various chlorinated organics which are then accessible to utilization by biota. This process leads to the production of trichloroacetic acid (TCA), which is considered to be an intermediate in mineralization of chlorinated soil organic compounds (Matucha et al.,

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2007). As such, TCA is a key chlorinated compound whose degradation rate may be taken as a measure of the microbial degradation of organochlorines in the soil.

The increase in chloride availability, affecting the composition of soil microbial cenosis, may thus affect the chlorine flux in the soil from its inorganic form to organic compounds and vice versa.

We tested the hypothesis that chloride concentration in the soil affects both microbial community and its chlorination and dehalogenation activities. We supposed that treatment of soil with high chloride concentration may select for multiplication of microorganisms involved in soil Cl cycling; this led us to the idea that increased concentration of soil chloride will increase the ability of the soil to degrade and produce chlorinated organic compounds. We studied also the effect of chloride on newly formed soil microbial community in irradiated soil, which may be more susceptible to soil treatment (chloride concentration) than already established community.

2. Materials and methods

2.1. Soil

Soil used in the experiment was O_H layer of podzol collected at the "Baně" locality (*Picea abies* monoculture), experimental station of the Forestry and Game Management Research Institute, Jíloviště Strnady near Prague. The soil was sieved through 2-mm sieve. Approx. 1.5 kg of the resulting material was washed in 5 l deionized water (conductivity 0.5 μS cm^{-1}) for 1 h at 25 °C to minimize the content of chloride. The soil was then retained on filter paper, left in open air at room temperature to dry up to 5.2% humidity and mixed. The chemical composition of the washed soil was analyzed using standard procedures of the Forestry and Game Management Research Institute (Pribyl, 2005). The results are shown in Table 1, second column. Known concentrations of mineral ions in crude, unwashed field-collected soil are given in Table 1, fifth column.

The soil was further divided into two equal portions, one of them being sterilized by 25 kGy dose of γ-radiation (the dose used for reliable sterilization of medical equipment) and inoculated with 1% (w/w) of the same nonsterilized soil. Nonsterilized as well as sterilized portion was then divided into six equal parts (108 g wet soil per each part), three sterilized and three nonsterilized parts were supplied with 20 ml of mineral solution (containing KH_2PO_4, $CaSO_4$, K_2SO_4, $MgSO_4$ and NaCl) per 108 g wet soil (representing one experimental replicate) in order to reach 20% humidity and the final ionic concentrations indicated in fourth column of Table 1. The same salts without NaCl were added in the same amounts to other three parts of nonsterilized soil and three parts of sterilized soil (Table 1, third column). In this way, three replicates were established per treatment. High chloride treatment soil contained 500 mg kg^{-1} chloride ions, which represents the increased chloride level common in soils along salted roads.

3. Analysis of microbial community using terminal restriction fragment length polymorphism

DNA was extracted from representative soil samples after 125 d of incubation according to Stach et al. (2001), purified using Geneclean® Turbo kit (Qbiogene) and stored at −20 °C until used.

Specific fragments had to be amplified from soil-extracted DNA using polymerase-chain reaction (PCR) before they could be subjected to analysis. PCR is a cyclic template-dependent DNA synthesis providing amplification (exponential increase in concentration) of a DNA fragment demarcated by two short nucleotide sequences. These two sequences are recognized by oligonucleotides called primers so that virtually any part of genome demar-

Table 1
Chemical analysis of crude soil, of experimental soil after washing and final concentrations of elements in experimental soil after supplying phosphate and chlorides to reach desired chloride concentrations in the treatments

	Washed soil	Washed soil + minerals	Washed soil + minerals	Crude (unwashed) soil
		Low Cl	High Cl	
pH (H_2O)	4.01	4.20	4.20	4.81
pH (KCl)	3.09	–	–	3.83
Oxidizable C (%)	20.0	20.0	20.0	31.8
Total C (%)	25.3	25.3	25.3	37.2
Total N (%)	1.07	1.07	1.07	1.54
Total S (mg kg^{-1})	1660	1660	1660	2110
Available P (mg kg^{-1})	32.0	100	100	212
Total Cl (mg kg^{-1})	*20.0*	*20.0*	*500*	*41.2*
Extractable Al (mg kg^{-1})	436	436	436	–
Extractable Ca (mg kg^{-1})	1853	1941	1941	4730
Extractable Fe (mg kg^{-1})	71.2	71.2	71.2	–
Extractable K (mg kg^{-1})	79.3	200	200	–
Extractable Mg (mg kg^{-1})	126	200	200	–
Extractable Mn (mg kg^{-1})	213	213	213	–
Extractable Na (mg kg^{-1})	17.3	17.3	332	–
Extractable Zn (mg kg^{-1})	19.2	19.2	19.2	–