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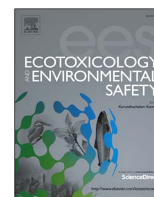

Salinity stress accelerates the effect of cadmium toxicity on soil N dynamics and cycling: Does joint effect of these stresses matter?

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ABSTRACT

The objective of this study was to determine responses of soil nitrogen (N) transformation, microbial biomass N, and urease activity to the combined effect of cadmium (Cd) toxicity (0 and 30 mg kg^{-1}) and NaCl stress (0, 7.5 and 15 dS m^{-1}) in a clay loam soil unamended (0%) or amended with alfalfa residues (1%, w/w). Cd, NaCl, and alfalfa residues were added to the soil, and the mixtures were incubated for 90 days under standard laboratory conditions (25 ± 1 °C and 70% of water holding capacity [WHC]). The results showed that salinity increased soil Cd availability and toxicity and subsequently decreased soil microbial N transformations (i.e., potential ammonification and nitrification as well as net N mineralization), arginine ammonification and nitrification rates, microbial biomass N, and urease activity. The adverse effects of salinity on soil microbial properties were greater in Cd-polluted than unpolluted soils, at high than low salinity levels, but were lower in residue-amended than unamended soils. These effects were mainly attributed to the increased Cd availability under saline conditions or the decreased Cd availability with residue addition. All the measured soil microbial attributes showed a negative correlation with the available Cd content in the soil. The interaction or combined effects of Cd and NaCl on soil microbial attributes were mostly synergistic in residue-unamended soils but antagonistic in residue-amended soils. The addition of organic residues to Cd-polluted soils may moderate salinity effect, and thus could stimulate the activity of ammonifiers and nitrifiers, as well as urease.

1. Introduction

Soil microorganisms play a critical role in key ecosystem processes and functions such as decomposition of soil organic matter (SOM) and organic wastes, carbon (C) sequestration, nutrient cycling and availability, and biodegradation of organic pollutants. These microbial processes are determinants and considered to be used for assessing soil fertility and quality because of their high sensitivity to environmental stresses or changes in management practices (Nannipieri et al., 2017). In soil, microbial ammonification, nitrification, and nitrogen (N) mineralization are important indicators of N transformation and cycling, as they directly regulate the availability of inorganic N forms to microorganisms and growing plants (Nannipieri et al., 2017). Environmental abiotic stressors such as salinity (Rietz and Haynes, 2003; Yuan et al., 2007; Rasul et al., 2006) and metal toxicity (Smolders et al., 2001; Dai et al., 2004; Belyaeva et al., 2005; Semerci and Cecen, 2007; Chaperon and Sauvé, 2007; Wang et al., 2017) are known to have a detrimental effect on the soil microbial communities and consequently on the microbially mediated processes and functions involved in N cycling and availability. Although, responses of soil microorganisms and biochemical properties to the single effect of salinity or metal toxicity is well established in the literature, to our knowledge, very little is known about how their combination affects the soil microbial communities and functions. Abiotic stresses including metal pollution, salinity, acidity, drought, waterlogging, compaction, freezing, warming, and pesticides often occur simultaneously or sequentially under realistic field conditions (Schimel et al., 2007; Holmstrupa et al., 2010; Suzuki et al., 2014; Schaeffer et al., 2017; Zheng et al., 2017). The joint effects of abiotic stresses would often differ from their single effects and may be stronger (synergistic, more than additive) or weaker (antagonistic, less than additive) than the expected effects from individual stresses (Folt et al., 1999; Suzuki et al., 2014).

Globally, both salinity and metal pollution are two major abiotic stresses, occurring concurrently or sequentially (Gabrijel et al., 2009; Abbas et al., 2017). NaCl salinity can enhance the solubility and mobility of toxic metals and thus their bioavailability in metal-polluted soils by affecting metal chemistry, speciation, and distribution (Ghallab and Usman, 2007; Gabrijel et al., 2009; Zhang et al., 2016). The increased metal mobility and solubilization under saline conditions (i.e., NaCl salinity) is predominantly ascribed to the formation of metal

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complexes with inorganic ligands (MCl_n^{2-n}) in the soil solution or competition between the Na^+ and metal ions for soil exchange sites (Usman et al., 2005; Ghallab and Usman, 2007; Du Laing et al., 2008; Abbaspour et al., 2008; Acosta et al., 2011; Chu et al., 2015; Zhang et al., 2016; Abbas et al., 2017). Chloride (Cl^-) salinity increases the mobility of metals such as nickel (Ni), copper (Cu), lead (Pb), and cadmium (Cd) in the soil solution owing to the formation of metal–chloride complexes (Ghallab and Usman, 2007; Abbaspour et al., 2008; Acosta et al., 2011). Consequently, it would be expected that saline irrigation water containing a high level of Cl^- ions would increase the adverse effects of Cd toxicity on microbial functions and properties in Cd-contaminated soils.

In the literature, much of the evidence for the interactive effects of salinity and metal pollution are mainly on plant responses, with an increased metal uptake by plants when grown in saline soils (Usman et al., 2005; Ghallab and Usman, 2007; Du Laing et al., 2008; Gabrijel et al., 2009; Zhang et al., 2016; Abbas et al., 2017). Yet, there is no adequate experimental information available to demonstrate the combined effects of metal and salinity, specifically on soil N mineralization, ammonification, nitrification, microbial biomass N, and urease (URE) activity. In a recent study, soil salinity increased Pb toxicity for URE activity in the coastal wetlands (Zheng et al., 2017). Increasing salinity levels lowered microbial respiration in a calcareous loamy sand soil polluted with Cd because of increased Cd mobilization and toxicity (Usman, 2015). Understanding the combined effect of abiotic stresses may provide predictions about how metal-polluted soils would respond to additional natural and anthropogenic stresses and how multiple stress factors would interact. This information is essential for managing stressors and protecting organisms (Breitburg et al., 1998; Schaeffer et al., 2017).

This research aimed to experimentally show the interactive effects of Cd toxicity and NaCl salinity on major N transformation processes, microbial biomass N, and URE activity involved in N cycling in a Cd-contaminated saline soil and whether these cumulative effects of Cd toxicity and salinity would vary with the addition of organic amendments under laboratory conditions.

2. Materials and methods

2.1. Soil collection

The experimental soil (0–20 cm depth) used for this study was collected from an uncultivated cropland located at Shahrekord University (32° 21′ 18.2″ N; 50° 49′ 29.9″ E, altitude 2085 m above sea level). The soil was nonsaline and unpolluted with clay loam texture, classified as Typic Haplocalcid. Soil samples were air-dried and passed through a 2-mm sieve. The soil had the following initial properties: Clay: 29%; silt: 42%; sand: 29%; $CaCO_3$: 35%; electrical conductivity (EC) of saturated paste extract (ECe): 1.3 $dS\,m^{-1}$; pH (1:2 ratio): 7.6; organic C: 0.57%; total N: 0.54 $g\,kg^{-1}$; total Cd: 1.65 $mg\,kg^{-1}$; available Cd: 0.15 $mg\,kg^{-1}$ and field capacity (w/w): 23%.

2.2. Experimental outline and incubation experiment

Experimental factors consisted of the following: (1) Cd addition (0 and 30 $mg\,kg^{-1}$ soil); (2) salinity [1.3 (as control), 7.5, and 15 $dS\,m^{-1}$]; and (3) alfalfa residue application (0% and 1%, w/w). The soil was polluted with the aqueous solution of cadmium chloride ($CdCl_2$) to obtain the above concentration. The air-dried soils and the added Cd were mixed thoroughly in large plastic jars. Deionized water was used to adjust soil water content at 70% WHC, and the jars were pre-incubated for 30 days to allow Cd distribution into soil matrix, reach an equilibrium condition, and reduce the effects of sampling and sieving disturbance. Saline water solution was prepared using the NaCl salt to obtain the desired EC levels. The NaCl and plant residue (1 mm, 1%) treatments were added at the end of preincubation period. The alfalfa residue contained 40% C and 3.2% N with a C/N value of 12.5. The control nonsaline soils were watered with distilled water and others with the corresponding saline solutions. The experiment was designed to expose a Cd-polluted soil to NaCl salinity mimicking the situations, where polluted soils are salinized following the use of saline water for irrigation.

2.3. Soil chemical and microbiological analysis

In the first experiment, subsamples (150 g) from the treated soils were placed into 1-l plastic jars. The jars were immediately pre-incubated at constant humidity (70% of WHC) and temperature (25 ± 1 °C) for 7 days. Nitrogen (N) mineralization potential was determined over a 90-day period using the method described by Alef and Nannipieri (1995). Soil net N mineralization was estimated from the increase of K_2SO_4 (0.5 M) extractable inorganic N (NO_3-N and NH_4-N) using a soil-to-extractant ratio of 1:4 before and after incubation. Initial and final inorganic N in the filtered extracts was analyzed colorimetrically (Alef and Nannipieri, 1995). The cumulative net ammonification and nitrification and the cumulative total N mineralized ($mg\,N\,kg^{-1}$ soil) in 90-day incubation were calculated.

In the second experiment, subsamples (500 g) from the treated soils were obtained and placed into 1-l plastic jars, and preincubated in a manner similar to that in the first experiment. Thereafter, soil arginine ammonification rate, nitrification rate, microbial biomass nitrogen (MBN), URE activity, DTPA-extractable Cd, pH, and EC were determined at days 30, 60, and 90. Potential arginine ammonification, a reflection of the activity of soil ammonifying microorganisms and N mineralization (Lin and Brookes, 1999), was quantified as described by Alef and Kleiner (1987). First, preincubated moist soil samples (2 g) were placed in centrifuge tubes and incubated for 30 min at 30 °C. Then, 0.5 ml of arginine solution (0.2%) was added to one tube and incubated further for 3 h at 30 °C. Distilled water (0.5 ml) instead of the arginine solution was added to the other tube and incubated at − 20 °C. Finally, 8 ml of KCl solution (1 M) was added to both centrifuge tubes and shaken for 30 min in a mechanical horizontal shaker. The samples were centrifuged for 10 min and filtered through a Whatman 42 filter paper. The NH_4^+ concentration was determined in the extracts colorimetrically at 660 nm (Alef and Nannipieri, 1995). The arginine ammonification rate was expressed in mg NH_4-N released g^{-1} dry weight h^{-1}. Nitrification rate, as an index for the activity of soil autotrophic nitrifiers, was determined (Alef and Nannipieri, 1995). The soil subsamples (10 g) were placed in Erlenmeyer flasks, and then, 1 ml of $(NH_4)_2SO_4$ solution (1%) was added. The samples were incubated for 3 weeks at − 20 °C (control) or 25 °C. After the incubation, soil samples were treated with 100 ml of $(NH_4)_2SO_4$ solution (1%) and flasks were shaken for 1 h. Soil suspension was then filtered through a folded paper filter. Soil extracts were immediately filtered, and the NO_3^- concentration was determined colorimetrically at 410 nm (Alef and Nannipieri, 1995). The size of soil MBN was determined following the fumigation–incubation method (Jenkinson and Powlson, 1976). Two 30-g fresh subsamples were placed in 50-ml glass beakers. One beaker was fumigated with ethanol-free $CHCl_3$ in a vacuum desiccator for 24 h at room temperature in the dark. The chloroform vapor in the desiccator was removed by repeated evacuations. Both fumigated and unfumigated soils were re-inoculated with 0.5 g of unfumigated soil. All beakers were placed in plastic containers with a plastic vial containing 10 ml of distilled water to keep soil moisture constant. The containers were closed tightly and incubated at 25 °C in the dark for 10 days. Inorganic N (NO_3-N and NH_4-N) was extracted by shaking 5 g of fumigated and unfumigated soil with 25 ml of 1 M K_2SO_4 solution for 30 min. Soil extracts were immediately filtered and analyzed colorimetrically for NO_3 and NH_4, as described above. The difference between the inorganic N released from fumigated and unfumigated samples was considered as MBN, using a standard factor of 0.57 (Jenkinson, 1988). The amount of MBN was expressed as $mg\,N\,kg^{-1}$

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