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Effects of NaCl and seawater induced salinity on survival and reproduction of three soil invertebrate species

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HIGHLIGHTS

- The effects of salinisation on soil ecosystems due to sea level rise were assessed.
- Soil invertebrates were exposed to NaCl and seawater in OECD soil.
- Increased sensitivity observed: *H. aculeifer* $\ll$ *E. crypticus* $\approx$ *F. candida*.
- Soil invertebrate sensitivity to NaCl depends from the exposure pathway.
- Adverse effects were found for soil conductivity values below the limit defined for saline soils.

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ABSTRACT

The increase of global mean temperature is raising serious concerns worldwide due to its potential negative effects such as droughts and melting of glaciers and ice caps leading to sea level rise. Expected impacts on soil compartment include floodings, seawater intrusions and use of saltwater for irrigation, with unknown effects on soil ecosystems and their inhabitants. The present study aimed at evaluating the effects of salinisation on soil ecosystems due to sea level rise. The reproduction and mortality of three standard soil invertebrate species (*Folsomia candida*, *Enchytraeus crypticus*, *Hypoaspis aculeifer*) in standard artificial OECD soil spiked with serial dilutions of seawater/gradient of NaCl were evaluated according to standard guidelines. An increased sensitivity was observed in the following order: *H. aculeifer* $\ll$ *E. crypticus* $\approx$ *F. candida* consistent with the different exposure pathways: springtails and enchytraeids are exposed by ingestion and contact while mites are mainly exposed by ingestion due to a continuous and thick exoskeleton. Although small differences were observed in the calculated effect electrical conductivity values, seawater and NaCl induced the same overall effects (with a difference in the enchytraeid tests where a higher sensitivity was found in relation to NaCl). The adverse effects described in the present study are observed on soils not considered saline. Therefore, the actual limit to define saline soils (4000 μS cm^{-1}) does not reflect the existing knowledge when considering soil fauna.

1. Introduction

Over the past years, the increase of global mean temperatures is causing the decrease of the snow cover and of the ice stocks. These events have originated a rising of the sea level (IPCC, 2007a) from an increase of 1.5 to 1.7 mm year^{-1}, observed in the last century, to an increase of 3 mm year^{-1} in the last decade (IPCC, 2013). The IPCC forecast for this phenomenon is between 40 and 62 cm until 2100 (IPCC, 2013) enhancing the risk of drought and flooding events (IPCC, 2007b). At a global scale, the most affected regions will be the arid and semi-arid parts of Australia, South America, Asia and Europe (European Soil Portal, 2012). In Europe, countries near the Mediterranean and Caspian Seas have been the most affected with an increase of 1 million hectares of saline soils in 2002 (Commission of The European Communities, 2002) to an estimated 3 million hectares in 2012 (European Commission, 2012). In Spain, about 3% of the 3.5 million hectares of irrigated land is affected by soil salinisation limiting the local agriculture (Van-Camp et al., 2004). Along with Spain, France, Greece, Italy and Portugal (among others) have extensions of 250, 3.5, 450, 25 thousands hectares of saline soils, respectively (Eckelmann et al., 2006). The rise of sea level will lead to soil salinisation mainly due to seawater (constituted by free ions of sodium – 31% – and chloride – 55%; Wiesenburg and Little,

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1987–1988) intrusions (IPCC, 2007b) and irrigation with saltwater (Wang and Li, 2012) since the freshwater availability and quality will be reduced (IPCC, 2007b). Besides being induced by global climate change, soil salinisation can also occur due to land irrigation with saltwater where a high evapotranspiration, higher temperatures and/or dry climate are found (leading to an increase of salt concentration in water and also the deposition of those salts on soils; IPCC, 2007b). The accumulation of soluble salts on soils negatively affects their fertility (European Soil Portal, 2012). Crop yields can be further affected due to the increase of pest species and/or diseases, risk of disappearance of less resistant species to salinity, with potential loss of biodiversity (IPCC, 2007b). Soil salinisation can occur through natural processes like the increased evapotranspiration (primary salinisation) and/or induced by human activities like the increase withdraws from aquifers (secondary salinisation) (European Soil Portal, 2012). The classification of saline soils depends not only on the amounts of salts dissolved, but also on the pH and the exchangeable sodium percentage (ESP). Soils with a pH lower than 8.5 and ESP lower than 15% are considered saline when the electrical conductivity (EC) is equal or higher than 4000 $\mu S\ cm^{-1}$ (Micheli et al., 2002).

Terrestrial and aquatic communities are differently affected by salinisation but all suffer a change on their species abundance and diversity with a dominance of salt-tolerant species (Davis et al., 2003; Andronov et al., 2012). Despite this, some species are known to tolerate salinity due to special morphological or physiological traits. For example, the spider *Arctosa fulvolineate* and the beetle *Merizodus soledadinus* can accumulate amino acids which originate an increase in the osmolality of body fluids (Foucreau et al., 2012; Hidalgo et al., 2013), while different species of amphipods can regulate the internal concentration of salts by their release in the urine (hypo-osmotic or isosmotic urine; Morritt, 1988).

Despite the existing knowledge on the impacts of salinity on coastal ecosystems in freshwater and plant species (James et al., 2003), soil organisms and their responses toward soil salinity have been neglected so far. From the few studies conducted, the most studied soil fauna groups are earthworms and nematodes. Besides the avoidance behavior to natural saline soils by earthworms (Owojori and Reinecke, 2009) and the complex relationship between nematodes and salt (with tolerance of *Caenorhabditis elegans* to salt in the presence of food and avoidance in its absence) (Adachi et al., 2010), effects in a long-term experiment are described for earthworms. Owojori et al. (2009) exposed two earthworm species (*Eisenia fetida* and *Aporrectodea caliginosa*) to a natural saline soil with electrical conductivities (EC) between 0.08 and 1.62 dSm^{-1} and found significant effects on growth, mortality and reproduction. Both species showed a higher sensitivity when considering reproduction (with the production of cocoons only in the control). In the same study, reproduction of springtails (*Folsomia candida*) and enchytraeids (*Enchytraeus doerjesi*) were also evaluated with significant effects on reproduction found. Complete cessation of reproduction was observed for springtails at 1.62 dSm^{-1} and for enchytraeids at 1.31 dSm^{-1} and above.

Effects like those reported above can impair soil functioning due to the relevant role that soil fauna has on key ecological processes like organic matter decomposition, nutrient cycling and maintenance of soil structure (Lavelle et al., 2006). Therefore it is essential to better comprehend salinisation impacts on soil fauna, to better perceive the effects of this stressor on soil functions underlying key ecosystems services.

The present study had three main objectives: (1) to evaluate the effects of exposure to sodium chloride (NaCl) and seawater on reproduction of three standard soil test-species; (2) to evaluate the use of NaCl as a surrogate of exposure of soil organisms to seawater and (3) to derive safety levels of salinity to soil fauna. In order to fulfill these aims, standard reproduction tests using three standard soil invertebrates (the springtail *Folsomia candida*, the enchytraeid *Enchytraeus crypticus* and the mite *Hypoaspis aculeifer*) were performed using artificial OECD soil spiked with a gradient of concentrations of salt (sodium chloride) or a gradient of seawater dilutions, the latter presenting equivalent electrical conductivity values to the former.

2. Materials and Methods

2.1. Test soil and concentration range for NaCl and seawater

The artificial OECD soil, used in all assays, was prepared mixing 5% of air dried and sieved sphagnum peat, 20% of kaolin clay and 75% of quartz sand. The pH was adjusted with $CaCO_3$ to 6.0 ± 0.5 (OECD, 2008). In order to evaluate the use of sodium chloride (NaCl) as a surrogate of seawater, two sets of tests were performed. A gradient of NaCl concentrations or seawater dilutions was used as shown in Table 1. NaCl concentrations were prepared diluting a stock solution of sodium chloride (Merck KGaA, 64271 Darmstadt, Germany) in distilled water. Seawater was collected from Praia da Barra in Aveiro, Portugal, filtered through cellulose nitrate membranes (0.20 µm) and kept at 4 °C until used. The NaCl gradient was prepared using a multiplication factor of 1.37 starting from 0.5 gKg^{-1} DW and ending at 4.5 gKg^{-1} DW (Table 1). The range of concentrations was performed based on the reproduction tests performed by Owojori et al. (2009) for springtails and enchytraeids. A different concentration range was used for the mite test due to the lower sensitivity observed in this species in the range finding test performed earlier (where no effects were observed on concentrations of up to 4.5 gKg^{-1} DW of NaCl). The seawater dilutions were prepared mixing seawater with distilled water. The concentrations of NaCl and the seawater dilutions were prepared in order to obtain an equivalent range of electrical conductivity (furthermore referred as conductivity) values. In case of mite tests, only six seawater dilutions were prepared since the equivalent conductivity values of the last three NaCl concentrations were higher than the conductivity of the soil when mixed with pure seawater (Table 1).

Table 1
Range of NaCl concentrations, seawater dilutions (SW) and the corresponding measured conductivity values used in the reproduction tests with *Folsomia candida*, *Enchytraeus crypticus* and *Hypoaspis aculeifer* (NaCl – salt concentrations; Cond – measured conductivity in the solution; SW – seawater dilutions; DW – dry weight.).

Folsomia candida and *Enchytraeus crypticus*	NaCl (g Kg^{-1} DW)	0	0.5	0.7	0.9	1.3	1.8	2.4	3.3	4.5
	Cond ($\mu S\ cm^{-1}$)	107.4	262	305	372	494	634	825	1057	1415
	SW (%)	0	5	8	10	14	19	25	33	45
	Cond ($\mu S\ cm^{-1}$)	134.5	309	388	456	615	742	988	1264	1677
Hypoaspis aculeifer	NaCl (g Kg^{-1} DW)	0	1.6	2.6	4.1	6.6	10.5	16.8	26.8	42.9
	Cond ($\mu S\ cm^{-1}$)	108.5	629	841	1276	1963	2760	4250	6830	10130
	SW (%)	0	17	24	38	59	91	100	–	–
	Cond ($\mu S\ cm^{-1}$)	180.5	729	997	1395	1973	3040	3320	–	–