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Combined effect of temperature and copper pollution on soil bacterial community: Climate change and regional variation aspects

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ABSTRACT

The aim of this study was to assess the combined effects of temperature and copper (Cu) contamination in the structure of soil bacterial community. For this, contaminated or spiked and control soils from two different geographic origins (PT-Portugal and DK-Denmark) were used. The DK soil was from a historically contaminated study field, representing a long-term exposure to Cu while the PT soil was from a clean site and freshly spiked with Cu. Soil bacterial communities were exposed in mesocosms during 84 days to 3 different temperatures based on values typically found in each geographic region and temperature conditions that simulated a warming scenario.

Obtained results indicate that Cu stress alters the structure of bacterial community and that this effect is, to some extent, temperature-dependent. Effects on bacterial diversity for both soils were also observed. Differences in the DK and PT communities' response were apparent, with the community from the historically contaminated soil being more resilient to temperature fluctuations.

This study presents evidence to support the hypothesis that temperature alters the effect of metals on soils. Further, our results suggest that the definition of soils quality criteria must be based on studies performed under temperatures selected for the specific geographic region. Studies taking into account temperature changes are needed to model and predict risks, this is important to e.g. future adjustments of the maximum permissible levels for soil metal contamination.

1. Introduction

The importance of microbial community in soil ecosystem functioning is well known, with bacteria being involved in a wide range of processes, e.g. nutrient cycling, organic matter decomposition or plant symbiosis (Burgin et al., 2011; van der Heijden et al., 2008). Such bacterial community is dependent on the temperature regime, and may change accordingly. It is also well known that high concentrations of chemicals, e.g. heavy metals, may cause detrimental effects on such bacterial community (Sheik et al., 2012; Zeng et al., 2007).

As metals are not degradable (although to some extent "inactivated"), these often impose a long term or permanent selective pressure on species. This pressure can result in changes in diversity, activity and biomass of bacterial community (Šmejkalová et al., 2003; Zhang et al., 2009). In particular, the species composition may be strongly altered since microbes are differently affected and a metal-tolerant community may be selected (Altimira et al., 2012; Giller et al., 2009; Piotrowska-Seget et al., 2005). The type and level of contamination determines the compositional shifts. Effects have been confirmed both on bacterial communities under laboratory and under natural conditions (Altimira et al., 2012; Ranjard et al., 2006). In the case of Copper (Cu), high concentrations are toxic to bacteria, imposing detrimental effects on cell metabolism (Magnani and Solioz, 2007). Moreover, also bacterial biomass reduction has been described to occur due to Cu exposure in soil (Wang et al., 2007), e.g. through Cu-mediated production of free radicals and oxidization of proteins and nucleic acids (Dupont et al., 2011). The sensitivity to Cu depends on the bacterial taxa e.g. Acidobacteria have been shown to be much more tolerant to Cu than Firmicutes (Wakelin et al., 2010).

Pollution-induced community tolerance (PICT) response is one of the most sensitive specific indicators of a community response to Cu (Brandt et al., 2010). Effects of long term exposure to Cu

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showed that the tolerance of the bacterial community to this metal generally increased with increasing Cu concentration but was best correlated with the bioavailable Cu fraction (Berg et al., 2012). Further, although no significant effect on Operational Taxonomical Unit (OTU) richness was detected, the researchers found that the bacterial community composition was highly impacted by Cu.

Little is known about the influence of metals on biological communities under changing temperatures. Due to global warming the mean air temperature at the Earth's surface is predicted to increase by 1.4–5.8 °C (IPCC, 2007), with extreme events occurring localised and more frequent than previous. The increase in temperature, and the extreme events, will affect organisms and ecological processes in both terrestrial and aquatic ecosystems. Almost certainly the global warming will induce shifts in the bacterial community composition, since species have different optimal temperature, hence giving competitive advantage to species adapted to higher temperatures (Castro et al., 2010). This, combined with higher temperatures, can lead to a change in metal uptake (and excretion) by bacteria (Rajkumar et al., 2013) and supports the hypothesis that Cu contamination and temperature increase may act synergistically, thus intensifying the selective pressure on soil bacterial community.

Hence, the aim of this work was to investigate the compositional modifications of bacterial community as imposed by different temperature regimes in uncontaminated soils, soils historically contaminated, and in soils recently spiked with Cu. In this context, we also aim to compare the effect on a Cu adapted versus non-adapted community, despite additional confounding factors. Effects were assessed within a mesocosms experimental setup and by Denaturing Gradient Gel Electrophoresis (DGGE) profiling of total bacterial community.

2. Materials and methods

2.1. The study sites

The experiments were conducted comparing two different geographic locations (1) Hygum, Jutland, Denmark (DK) and (2) Aveiro, Portugal (PT).

The soil from DK (N 55°47′0″ W 9°26′0″) represents a historically Cu-contaminated site with a known Cu-gradient and history. Records go back to 1911, where a timber Cu impregnating factory operated until 1924; from 1924 to 1991 the area was used for agriculture purposes and thereafter, it was permanently fallow (Pedersen et al., 1999). At present, Cu concentration in soil ranges from background levels of ca. 20 mg/kg to 2911 mg Cu/kg soil (dry weight), with a gradual increase toward the centre of the field (Pedersen et al., 1999; Scott-Fordsmand et al., 2000; Strandberg et al., 2006).

The soil from PT represents a soil without any prior contamination history. The field study is located at the University of Aveiro, 200 m from the Ria de Aveiro (N 40°37.538′ W 008°39.691′). The site occupies a total of approximately 1600 m² and has not been used for agriculture or other purposes for at least 30 years.

2.2. Test soils and procedures

Soil samples (upper 0–20 cm) were collected in both sites, including the Ct and Cu-contaminated Hygum area with the concentration of 1000 mg Cu/kg (as confirmed using Atomic Absorption Spectroscopy). Soil characteristics are summarised in Table 1 for the DK and PT soils.

Soil from PT was used as control and spiked with Cu prior experiments to 100 mg Cu/kg (AAS confirmed). Samples will be referred as PT-Ct (Portuguese soil as collected from field) and PT-Cu (Portuguese soil spiked with $CuCl_2$). The copper salt $CuCl_2 \cdot H_2O$ (99% purity, Merck Pro Analysis, Darmstadt, Germany) was used. Spiking was done with $CuCl_2$ as an aqueous solution into pre moistened soil batches. The procedure consisted on dividing the soil into amounts of 4 kg dry weight to make it easily mixed. The mixing was manually performed during 30 min per batch.

Collected soils were first defaunated and then the microbial community was restored by inoculation with controlled microbial substrate from the respective locations. To exclude soil fauna, mainly invertebrates, the soil was dried at 80 °C during 24 h in an oven (Memmert, Type UL40, Braunschweig, Germany), as recommended for standardization purposes, and then sieved through a 2 mm mesh to remove larger particles. The procedure to extract the microbial substrate and inoculate the soil was based on Scott-Fordsmand et al. (2008) as follows: freshly collected soil from each of the control areas of the Hygum and Aveiro for the DK and PT field sites (1 kg wet weight) was mixed with 2 L of deionized water and shaken for 3 h. After this, the soil–water solution was filtered through a 50 µm mesh and diluted 10 times to use in the experiment. The soil water content was adjusted to 20% of which 10% was carried with the microbial substrate. This aimed to ensure comparable conditions between soils/regions.

2.3. Mesocosms experimental conditions

The soil samples for microbiology analysis were part of a full mesocosms multi-species experiment (full details in Menezes-Oliveira et al., 2013, 2014). In short, test chambers consisted of a polyethylene tube (33 cm × 9.3 cm ø), each unit containing 1000 g soil. To each container 6 invertebrate soil species were added at test start. Experiments ran in controlled temperature (± 1 °C) rooms. Each unit was covered by a perforated lid, weighted and stored at the correspondent temperature with a 12:12 h light:dark cycle. The soil water content was kept the same by replenishing with deionised water to the soil surface.

For analysis, soil samples were collected from three layers of three replicate mesocosms per condition. Test temperatures were 10–14–20–23 °C for the DK soil and 20–23–26–29 °C for PT soil. Temperature range was selected based on the regional variation on soil temperatures per year (ca. min–max of 0–19 °C and 5–25 °C for DK and PT respectively). Samples were collected at 4 exposure periods: 0, 28, 61 and 84 days.

Analyses were performed following a selection strategy. In this way the following stepwise approach was adopted:

1. Samples from the three mesocosms layers (T: Top; M: Middle; B: Bottom) were studied to assess differences between layers.

Table 1
Summary of the characteristics of the test soils (DK for Denmark and PT for Portugal) in terms of grain size distribution (%), pH (H_2O) and Organic Matter (OM) content (%).

Soil site	pH (H_2O)	OM (%)	Coarse sand (%; > 200 µm)	Fine sand (%; 63–200 µm)	Silt (%; < 63 µm)	Clay (%; < 2 µm)
Dk	6.3	4.5	20–32	20–25	11–20 (20–63 µm) 12–20 (2–20 µm)	12–16
Pt	5.3	3	37	11.2	35	_