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Soil microbial and physical properties and their relations along a steep copper gradient

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ABSTRACT

Copper (Cu) is accumulating in agricultural soils because it is an essential component of mineral fertilizers and pesticides. This could lead to toxic effects on soil macro- and micro-organisms and impact soil structure development. We investigated the effect of historical Cu contamination (>80 years; from background concentrations up to 3837 mg Cu kg^{-1}) on soil microbial enzyme activity, physical properties and resilience to compression. Soil samples and cores were taken from a fallow sandy loam field in Denmark. Microbial activity was quantified using fluorescein diacetate (FDA) and dehydrogenase (DHA) assays. Water dispersible clay was measured on field moist and air dried samples. For the resilience assay, soil cores (drained to −100 hPa) were subjected to uniaxial confined compression (200 kPa) followed by wet–dry or freeze–thaw cycles. Microbial enzyme activity significantly decreased with Cu concentration $\gtrsim$500 mg kg^{-1} with the two microbial assays linearly correlated with each other as well as with the water dispersible clay. An effect concentration causing a 50% reduction (EC_{50}) in enzyme activity was observed at 521 mg kg^{-1} for FDA and 542 mg kg^{-1} for DHA. Significant increases in water dispersible clay, bulk density and decreases in air-filled porosity and air permeability were observed from Cu $\gtrsim$ 900 mg kg^{-1}. The increased density of the contaminated soils led to greater compression resistance and resilience relative to the uncontaminated soil. The results suggest that a threshold level for Cu exists (~500 mg kg^{-1} for this soil type) beyond which microbial activity decreases and soil structure becomes more compact with reduced permeability to air.

1. Introduction

The addition of contaminated waste materials, mineral fertilizers, and pesticides increases the concentration of heavy metals such as copper (Cu) in agricultural soils. Although small amounts of these heavy metals are required for essential processes in living organisms, elevated levels are toxic to soil microbes and other organisms (Bååth, 1989), causing a detrimental effect on all soil processes in which they are involved. However, the age of contamination is an important variable in ecotoxicological studies (Tom-Petersen et al., 2004). Soil contamination from recent pollution or laboratory spiking has higher toxicity to soil microorganisms and plants than historically contaminated fields due to the effect of ageing on toxicity (Ma et al., 2006). Microbial enzyme activity in soils is important for processes such as mineralisation and transformation of organic carbon and plant nutrients (Burns, 1982). Pollutants affecting enzyme activity have an effect on both soil development and plant growth (due to the role of enzymes in decomposition of plant litter and roots). As a result, quantification of enzyme activity using assays such as dehydrogenase (DHA) and fluorescein diacetate (FDA) hydrolysis is done to determine the effects of contaminants on soil microorganisms (Xie et al., 2009). The effect of a specific heavy metal on soil microbial populations and associated processes is often difficult to isolate in field studies because most polluted sites have a mixture of heavy metals (Spurgeon and Hopkin, 1995). Studies involving single-pollutants are often conducted in the laboratory where spiked soil samples are used making it difficult to extrapolate to field scenarios. A fallow sandy loam field located at Hygum, Denmark offers a unique opportunity to study the effect of a single pollutant (Cu) on soil properties (physical and biological). For this field, earthworms and enchytraeids were adversely affected beyond Cu concentrations of 200 mg kg^{-1} (Maraldo et al., 2006; Holmstrup and Hornum,

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2012), whereas Collembola (*Folsomia fimetaria*) were not affected at Cu concentrations as high as 2900 mg kg^{-1} for field contaminated soils (Scott-Fordsmand et al., 2000). Plant biomass, species richness and vegetation height also decreased with increasing Cu content with plant community composition significantly changing at Cu concentrations >200 mg kg^{-1} (Strandberg et al., 2006). The development of soil structure results from microbial activity, plant roots proliferation and penetration, wetting and drying cycles, and activities of oligochaete worms (earthworms and enchytraeids) in combination with organic and inorganic cementing agents (Oades, 1984). Hence, contamination of soils with Cu indirectly affects soil structure development by disrupting activity of microorganisms (reduced organic material breakdown), soil fauna, and reducing plant growth. Rapid soil structure quantification involves measuring properties such bulk density, aggregate strength and stability, and transport of water and gases (Six et al., 2000; Moldrup et al., 2001). Quantifying the state of soil structure is not enough for environmental policy makers, it is also important to identify how the structure responds to common stresses it is exposed to (e.g. mechanical stresses due to tillage and other operations).

Interestingly, studies evaluating soil contamination effects often focus on biological activity (as shown in references above for Hygum as well). However, to have a holistic view of the impact of anthropogenic contaminants on the environment, all three components of soil quality (biological, chemical and physical) need to be linked (de Jonge et al., 2009). Also, results obtained should fit into a broader soil ecosystem framework which will serve as a decision-support tool for policy makers (Robinson et al., 2012). Considering the wealth of information on the identified Cu transect in Hygum, we embark on the first interdisciplinary study on the possible effects of elevated Cu levels on soil ecosystem functions by applying basic soil physical, mechanical and biological indexes. We hypothesise that elevated Cu levels resulting from historical contamination decreases microbial enzyme activity (biological) and result in a more compact soil structure with weaker aggregates (physical) and decreased resilience to soil compression (mechanical). To test this hypothesis, the objectives were to:

(a) Examine how elevated concentrations of copper resulting from historical contamination affects microbial enzyme activity, soil physical properties (e.g. bulk density, clay dispersion and pore organisation) and soil resilience to mechanical stress.
(b) Identify the Cu concentration level beyond which these effects become significant.

2. Materials and methods

2.1. Study site

The experimental site (7200 m^2), situated at Hygum (55°46′N, 9°27′E), Denmark, is an abandoned agricultural field contaminated with copper sulphate used for timber preservation operations between 1911 and 1924. Between 1924 and 1993, annual ploughing and harrowing during agricultural operations led to a homogenous mixing of the Cu in the upper 25 cm of the soil. Since 1993, the field has been under fallow (natural vegetation) with no further tillage. Further information about the site is given in Strandberg et al. (2006) and Holmstrup and Hornum (2012).

2.2. Sampling and measurements

2.2.1. Sampling

Sampling was done at seven selected areas in the field representing a gradient in increasing Cu-concentrations. The sampling along this gradient was based on a previous study (Holmstrup and Hornum, 2012). The average distance between the sampling areas was 15 m and the size of each sampling area was 0.5 m^2. From each sampling area, seven intact soil cores (100 cm^3, 6.1 cm diameter, and 3.4 cm height) were taken from a depth of 5–10 cm using a special flange placed on a sharpened steel sample ring and the latter driven into the soil by gentle hammering. Afterwards, bulk soil was taken from the same area using a shovel. Care was taken to avoid smearing or compaction of the soil on its way to the laboratory. At the laboratory, the bulk soil was gently spread out in a 20 °C room to air-dry. During the drying process, the larger, clods/aggregates were gently broken into smaller pieces to facilitate the process. The soil was then mechanically crushed and sieved to <2 mm for further analyses.

2.2.2. Basic soil physical and chemical properties

Soil texture was determined on <2 mm sieved soil by a combination of wet sieving and hydrometer methods (Gee and Bauder, 1986). Total carbon was determined on ball-milled sub-samples by oxidation of carbon to CO_2 at 1800 °C using FLASH 2000 organic elemental analyser coupled to thermal conductivity detector (Thermo Fisher Scientific, MA, USA). Soil Organic matter was estimated as total carbon content multiplied by a factor of 1.724 (Nelson and Sommers, 1996).

Total Cu concentration was determined using hot plate extraction in nitric acid as previously described (Pedersen et al., 1999). Briefly, 0.3 g dried soil was sifted through a 2 mm mesh and crushed in a mortar. Two ml of 7 N HNO_3 *pro analysis* (Merck, Damstadt, Germany) was added followed by heating to 80 °C for 17 h, and finally the fluid was heated to 110 °C until dryness. Another 1 ml 7 N HNO_3 was added to each sample and the procedure was repeated. The samples were dissolved in 5 ml 0.1 M HCl and then analysed by Atomic Absorption Spectrometry (AAS, Perkin Elmer 4100, Ueberlingen, Germany). A certified reference soil (VKIJ1, Danish Hydraulic Institute, Denmark) was included as an external standard. Extraction with 0.01 M $CaCl_2$ was used as a measure of the available Cu fraction (Novozamsky et al., 1993). Twenty ml 0.01 M $CaCl_2$ was added to 2 g dry, sifted soil and the sample was shaken end-over-end for 20 h, and then centrifuged at 5000 rpm for 5 min at room temperature (Fischer Scientific, Osterode, Germany). The supernatant was used for Cu analysis with AAS as previously described (Pedersen and van Gestel, 2001).

For soil pH, 8 ml of air dried soil was taken and 30 ml of deionised water added. The mixture was mechanically shaken for 10 min and left to settle for another 10 min. An electrode was then used to measure soil pH (Page et al., 1982).

2.2.3. Microbial activity

Microbial activity was estimated for air-dried aggregates in triplicates using two methods: fluorescein diacetate (FDA) [3′,6′-diacetylfluorescein] hydrolysis and dehydrogenase activity (DHA) by iodonitrotetrazolium reduction.

Fluorescein diacetate hydrolysis activity was determined as described by Schnürer and Rosswall (1982). Briefly, 4 × 1.5 g of 2 mm-sieved soil was mixed with 20 ml of sodium phosphate buffer 60 mM, pH 7.6 in glass tubes, and the reaction was started with the addition of 100 μl 5.0 mM FDA. After 2 h incubation with mechanical shaking, the reaction was stopped by adding 3 ml of acetone. The tubes were centrifuged for 5 min at 3000 rpm and the absorbance read at 490 nm. FDA hydrolytic activity was expressed as μg fluorescein g^{-1} soil h^{-1}. Dehydrogenase activity was determined by reduction of 2-p-iodo-nitrophenyl-phenyltetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF) following the method of García et al. (1993). Briefly, 6 × 1.5 g of soil was weighed into tubes placed on ice and 0.75 ml potassium phosphate buffer (0.2 M, pH 7.5) added. One ml of INT solution (0.4% INT in distilled water) was added and the tubes incubated in the dark at 20 °C for 4 h.

Five ml of analytical grade ethanol was added and shaken for 10 s. The solution was filtered through a 0.7 μm filter into cuvettes. The absorbance was measured at 485 nm and DHA expressed as μg INT g^{-1} soil h^{-1}.

2.2.4. Clay dispersion and soil pore characteristics

The water dispersible clay content (WDC) was determined in triplicate on 1–2 mm air-dried aggregates and field moist samples using the end-over-end shaking method (Schjønning et al., 2002). Briefly, a mixture of 10 g of the aggregates and 80 ml of artificial rainwater (0.012 mM $CaCl_2$, 0.15 mM $MgCl_2$ and 0.121 mM NaCl; pH 7.82; EC 2.24×10^{-3} S m^{-1}) was placed on a rotating shaking device (diameter 213 mm; rotation speed ~33 rpm) for 2 min. After shaking, the samples were removed and left undisturbed for sedimentation for 230 min. Afterwards, the top 60 ml of the suspension corresponding to the particles <2 μm (according to Stokes' Law) was transferred into a beaker. 10 ml of the suspension was then transferred to a pre-weighed glass vial followed by oven drying at 105 °C. The weight of dispersed colloids was determined on dry-weight basis for both air-dried and field-moist samples (mg clay g^{-1} dry soil).

Soil total porosity, Φ, was estimated from measured bulk density, ρ_b, and particle density, ρ_s, determined by the pycnometer method (Flint and Flint, 2002a). Soil volumetric water content, θ, at −100 hPa matric potential was taken as the respective difference in weight of the equilibrated samples and oven-dried samples multiplied by ρ_b. Total air-filled porosity, ε, was calculated as the difference between Φ and the θ (Flint and Flint, 2002b).

The effect of long term Cu pollution on the convective flow of gases and configuration of the pore system was examined by measuring the air permeability, k_a, and soil pore organisation (k_a/ε), at −100 hPa matric potential. The k_a was measured by the steady state method described by Iversen et al. (2001).

2.2.5. Soil compression and estimation of resistance and resilience

The resistance and resilience of the soil cores to uniaxial confined compression was investigated using k_a, void ratio, e, and ε as functional indicators of structural resistance and recovery. After equilibration of the soil samples at matric potential of −100 hPa, the weight and height, H, (using a specially constructed calliper with 6 replicate measurements) of the soil cores were determined to enable the calculation of the initial void ratio, e_0, using Eq. (1)

$$\text{Void ratio} = \left[\frac{\rho_s H - d}{\rho_b H}\right] - 1 \tag{1}$$

where d is the displacement of the soil in cm after compression. For e_0, $d = 0$.

The compression test involved subjecting soil cores to uniaxial confined compression to a maximum load of 200 kPa at a constant rate of 2 mm min^{-1} (Koolen, 1974) followed by unloading at the same rate. This compression stress simulates that imparted by agricultural machinery (Lamandé and Schjønning, 2008). The weight, d and k_a of the soil were measured immediately after compression. Afterwards, four of the seven cores were subjected to two wet–dry cycles, comprising −5 hPa on a sandbox for 24 h and 40 °C for 24 h followed by equilibration at −100 hPa. The remaining three cores were subjected to two freeze–thaw cycles comprising freezing at −10 °C for 24 h and re-equilibrating at −100 hPa on a sandbox. The freeze–thaw and wet–dry cycles were repeated once because the d and k_a of the soil did not differ significantly ($p > 0.05$ by Student's t test) between the two cycles. After the cycles, all soil cores were oven-dried at 105 °C for 24 h. Functional resistance (RS) and resilience (RL) were estimated using Eqs. (2) and (3), respectively.

$$\text{RS} = 100 - \left[\frac{|C_0 - D_c|}{C_0}\right] \times 100 \tag{2}$$

$$\text{RL} = \left[\frac{|D_x - D_c|}{D_c}\right] \times 100 \tag{3}$$

where C_0 is the original (unstressed) value, D_c is the value immediately after compression and D_x is the value following wet–dry or freeze–thaw cycles. For a given variable, the RS index is bounded by 0 (no resistance) to 100 (full resistance); the RL index is bounded by 0 (no resilience) to an indefinite maximum (but interpretable as a percentage of the stressed situation).

The compression index, C_c, was used to estimate the general resistance to compaction. The estimation of C_c is briefly explained below:

First, the soil compression data (applied stress, σ, and e) obtained was fitted to the Gompertz (1825) equation using non-linear least squares fitting (Gregory et al., 2006):

$$e = a + c\ \exp[-\exp(b(\log_{10}\sigma - m))] \tag{4}$$

where a, b, c and m are fitted parameters. The value of a corresponds approximately to the lower (final e) asymptote, while $a + c$ is the upper (initial e) asymptote. The compression index (C_{c^*}) was estimated as the modulus of the slope at the inflection point ($\log_{10} \sigma = m$) as defined by Gregory et al. (2006):

$$C_{c*} = \frac{bc}{\exp(1)} \tag{5}$$

Since the estimation of C_{c^*} could be erroneous when the inflection point is outside the range of the measured data (Keller et al., 2011), m was restricted to $m \le \log_{10}$ 200 kPa = 2.305.

2.3. Statistics

For comparison of microbial activity and soil properties resulting from the copper gradient, the Kruskal–Wallis non-parametric test in SPSS 19 (SPPS Inc., Chicago, USA) was used to test for significant differences ($p < 0.05$) between the means of all variables for the different Cu levels. This test was used because it does not assume normality in the data and is much less sensitive to outliers. When significant differences occurred among the different Cu levels, the Mann–Whitney U test was used to differentiate between the means. The Gompertz model was parameterised using the nonlinear regression analysis (solver) feature in Microsoft Excel. Other linear and non-linear regression model tests were conducted with SPSS 19. To obtain the effect concentration causing a 50% reduction in microbial enzyme activity (EC_{50}), data from both assays was normalised against enzyme activity in the control and the data fitted to a four-parameter logistic model using SigmaPlot version 11 (Systat Software, Inc., San Jose – CA, USA).

3. Results and discussion

3.1. Basic soil characterisation and the copper gradient

The soil texture of the Hygum field was a sandy loam, with similar clay contents across the seven study areas. However, higher silt and lower sand contents were observed for the sampling areas with high Cu levels. The total Cu concentration increased from background levels of 21.5–3836.7 mg kg^{-1}. The $CaCl_2$ extractable Cu fraction was linearly correlated to the total Cu concentration ($R^2 = 0.99$) for the field as reported previously (Pedersen and

Table 1
Basic soil characteristics.

Total Cu (mg kg^{-1})[a]	$CaCl_2$ Cu (mg kg^{-1})[a]	Clay < 2 μm (kg kg^{-1})	Silt 2–50 μm (kg kg^{-1})	Sand 50–2000 μm (kg kg^{-1})	Organic matter (kg kg^{-1})	Soil pH–H_2O (kg kg^{-1})[a]
22 ± 1	0.17 ± 0.01	0.11	0.22	0.64	0.033	6.1 ± 0.04
48 ± 4	0.48 ± 0.02	0.11	0.23	0.62	0.035	6.2 ± 0.01
175 ± 6	1.64 ± 0.11	0.10	0.22	0.65	0.034	5.9 ± 0.07
466 ± 18	5.27 ± 0.27	0.11	0.27	0.58	0.037	6.2 ± 0.07
875 ± 21	8.97 ± 0.46	0.12	0.29	0.54	0.042	6.2 ± 0.01
2228 ± 83	26.42 ± 2.30	0.11	0.36	0.48	0.051	6.3 ± 0.02
3837 ± 158	49.50 ± 4.45	0.12	0.28	0.57	0.027	6.6 ± 0.00

[a] Mean values ± standard error (n = 7).

van Gestel, 2001). Due to this correlation, the total Cu concentration was used throughout the paper. The mean soil pH–H_2O was 6.2 and showed no significant variation between the study areas. The content of soil organic matter (OM) increased with increase in Cu except for the highest Cu concentration (Table 1).

3.2. Effect of copper contamination on microbial properties

Total soil microbial activity quantified by FDA and DHA along the Cu concentration gradient is shown in Fig. 1. The FDA showed a bell-shaped response to increasing Cu concentration up to a threshold concentration of 466 mg kg^{-1} after which it decreased significantly with increasing Cu concentration. Similarly, significant decreases in DHA were observed at concentrations above 466 mg kg^{-1}. A similar decrease in DHA with increasing Cu content has been reported by Kizilkaya et al. (2004). The two indicators (FDA and DHA) were linearly correlated (R^2 = 0.88; p < 0.05) with similar EC_{50} values (521 ± 23 mg kg^{-1} for FDA and 542 ± 1.5 mg kg^{-1} for DHA). It has, however been suggested that presence of Cu in soils interferes with the measurement of DHA in soils, since other indicators (e.g. biomass C, biomass specific respiration) in the same experiments showed a significantly lower decrease in microbial activity than DHA (Chander and Brookes, 1991; Obbard, 2001). It must be noted however, that in the study of Chander and Brookes (1991), soils were artificially spiked in the lab and observations made over a period of 7 days. Also, the study of Obbard (2001) did not include soils and may be too simplistic for extrapolation to field conditions. For the present study, the DHA assay was used because (i) the contamination occurred 85 years ago, (ii) it includes soil, and (iii) Current research (Chaperon and Sauvé, 2008; Fernández-Calviño et al., 2010) have shown that enzyme activity measured using DHA in Cu-contaminated soils is comparable to other assays (urease, β-glucosidase, and phosphatase) This, however, does not nullify the findings of Chander and Brookes (1991) and Obbard (2001) as far as laboratory/greenhouse spiked experiments are concerned.

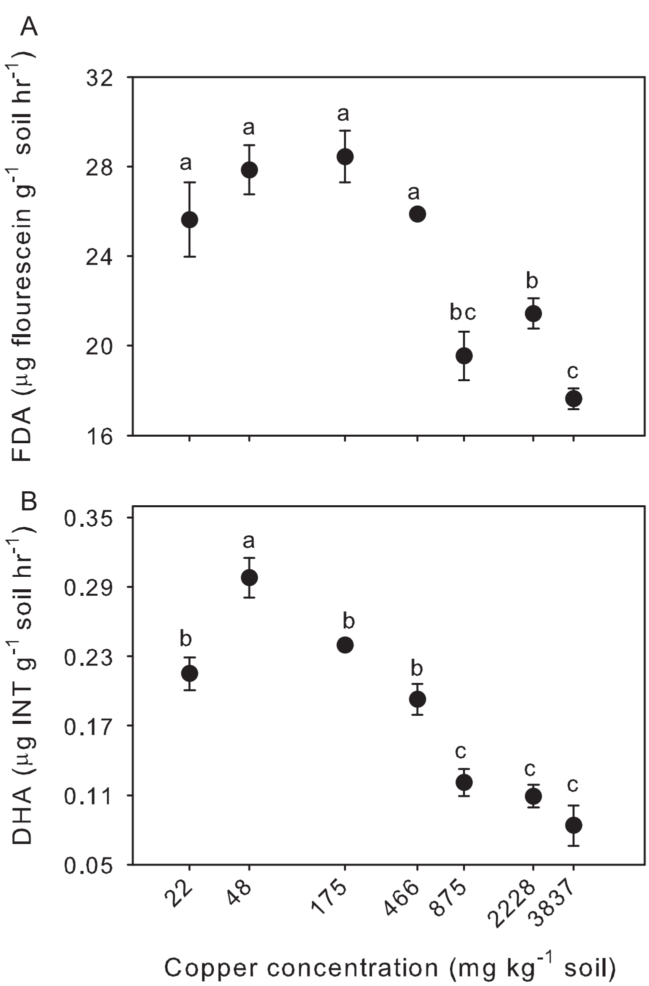

Fig. 1. Effect of copper concentration on microbial activity (indicated by flourescein diacetate, FDA hydrolysis and dehydrogenase activity, DHA). Bars indicate standard error of the mean (SEM). Differently lettered means are significantly different at p < 0.05.

The effect of a specific heavy metal on microorganisms is affected by soil pH (through speciation and solubility of metals), organic matter (through chelation), presence of other ions (interfering with uptake) and soil texture (clay reduces availability by chelation) (Giller et al., 1998). In the present study, effects of pH and interfering ions can be ruled out since all the areas had similar pH values (Table 1) and have background concentrations of other heavy metals (Holmstrup and Hornum, 2012). The higher OM of the high Cu concentration areas would ideally increase complexation and reduce the amount of bioavailable Cu ions. However, the extreme concentrations of Cu appear to negate any significant effect that OM alone would have on the level of toxicity as can be seen in Fig. 1. Further, the strong correlation between total and $CaCl_2$-extractable Cu concentration (R^2 = 0.99; p < 0.01) suggests that OM in this soil type has little influence on Cu toxicity. Low levels of microbial activity observed for the high OM areas (with high Cu) must therefore be due to strong effects of Cu. The increase in microbial activity from background levels to 175 mg kg^{-1} could be because microfauna (protozoa and nematodes) that feed on bacteria are more sensitive to Cu than bacteria. As a result, bacteria have an advantage at intermediate Cu concentrations until Cu levels become so high that toxic effects overshoot the benefits from reduced grazing by microfauna. Microarthropods from the same field also showed a similar trend: having higher numbers at intermediate concentrations (Pedersen et al., 1999). Brandt et al. (2010), using a maximum Cu concentration of 500 mg kg^{-1} reported that the soil bacterial community was resistant to a five-year field exposure to Cu. This may explain why significant decreases in microbial activity were observed only above Cu concentrations of 466 mg kg^{-1}. However, the continuous, though not statistically significant, decrease in microbial activity after 875 mg kg^{-1} can be attributed to the decreased enzyme synthesis resulting from Cu toxicity which is associated with inhibited microbial growth (Bååth, 1989).

When considering soils with identical geological origin, climatic conditions and management practice, the OM content depends on the balance between input and decay (turnover). The increase in OM with increasing Cu concentration (until 2228 mg kg^{-1}) together with the decreasing biological parameters (FDA and DHA) can be interpreted as an inhibition of microbial turnover of OM input leading to reduced decomposition of dead plant material (Sauve, 2006). The low OM of the area with the highest Cu concentration is due to reduced plant input emanating from low plant biomass (Strandberg et al., 2006). This is confirmed by the fact that when agricultural activities ceased on the field in 1994, plant biomass was largely the same with the exception of the hot spot. Currently, woody plants are more concentrated in intermediate Cu concentrations and could also explain the higher OM contents (Holmstrup and Hornum, 2012).

3.3. State of soil structure after copper contamination

A history of Cu concentration resulted in increased bulk density, significant at Cu concentrations >875 mg kg^{-1} (Table 2). This increase, coupled with decreased particle density (data not shown) also led to similar trends in the void ratio which generally decreased with increasing Cu concentration (Table 2). Soil total porosity also showed decreasing trends, with significant decreases also observed after 875 mg kg^{-1}. The volumetric water content, θ was similar for areas with Cu concentration <875 mg kg^{-1} followed by a significant increase in the last two areas with the highest concentrations. The higher θ for the area with 2228 mg kg^{-1} concentration can be explained by the greater OM content; this is however, not the case for the most polluted area which had the least OM content.

Generally, increasing OM content is associated with decreasing bulk density and higher soil total porosity (Ekwue, 1990). Seemingly contradictory results obtained here may be attributed to lower microbial activity (Fig. 1), decreased plant cover and biomass (Strandberg et al., 2006) and decreased earthworm activity (Holmstrup and Hornum, 2012). Studies conducted on the same field (and along the very same gradient) showed that earthworm total density decreased from 376 m^{-2} where Cu was at background levels to 24 m^{-2} in the area having Cu concentration of 2227 mg kg^{-1}, and no earthworms at all in the area with highest Cu concentrations (Holmstrup and Hornum, 2012). Specifically, the dominating endogeic and anecic earthworm species of the Hygum site (*Aporrectodea longa*, *Aporrectodea tuberculata* and *Aporrectodea rosea*), which generally increase the number of soil pores due to their burrowing activities (Lee, 1985; Lamandé et al., 2011), disappeared when Cu concentrations exceeded 300–500 mg kg^{-1} (Holmstrup and Hornum, 2012). The disappearance of these earthworm species and lowered plant root biomass are likely contributing to the increasing bulk density and decreasing total porosity at higher Cu concentrations. Another previous study on soil biota at Hygum has shown that enchytraeid (small white earthworms) abundance and biodiversity decreased when Cu concentrations exceeded ~500 mg kg^{-1} (Maraldo et al., 2006). Although the effects of enchytraeids on soil porosity are much smaller than the effects of earthworms, these organisms may have influence on soil microbial processes and formation of microaggregates (Didden, 1990).

An important measure of soil structural stability is the amount of water-dispersible clay (WDC) in soil aggregates. The WDC of 2 mm-sieved aggregates increased consistently with increasing Cu concentration for field moist samples (Fig. 2a), whereas for the air dried samples, significantly higher WDC was observed for only the last two areas with the highest Cu concentrations (Fig. 2b). Higher

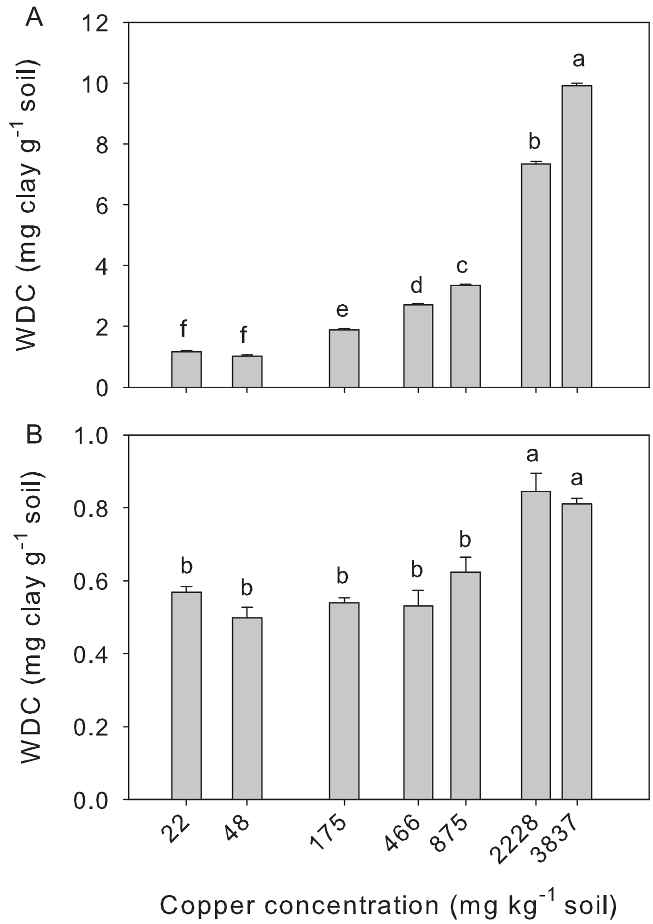

Fig. 2. Water dispersible clay, WDC, as affected by copper concentration for (A) field moist and (B) air dried aggregates. Bars indicate SEM. Differently lettered means are significantly different at $p < 0.05$.

WDC reflects weaker soil aggregates that are more susceptible to breakdown. Relocation of dispersed colloids (resulting from higher aggregate breakdown) may fill existing soil pores, increase bulk density and lower air and water permeability (Dexter, 1988). Upon drying, dispersed clay reduces soil friability as a result of cementation (Kay and Dexter, 1992). The WDC is largely influenced by clay content (Vendelboe et al., 2012), OM (Czyz et al., 2002), short-term management effects (Schjønning et al., 2002) and water content (Kjaergaard et al., 2004). Several studies have shown that air-drying of soil aggregates may introduce changes in chemical and physical characteristics that can influence the WDC (Reid and Goss, 1981; Dexter et al., 2011). However, conclusions drawn from results from only field moist samples may be complicated by the effect of water content (when samples have different field water contents) and the inclusion of air-dried samples may help reduce this discrepancy. The similar clay contents of the study areas makes the influence of the clay content negligible [R^2 = 0.22 (field moist); 0.15 (air dried)]. Additional regression analyses showed that soil organic carbon (OC) and the clay/OC ratio were not significant determinants of WDC in this study [R^2 = < 0.1 (OC) and <0.15 (clay/OC) for both moisture contents]. This leaves soil water content and Cu as the two factors with possible major effects on the WDC. Regression analyses using total Cu content always yielded higher correlation values than when using the $CaCl_2$-extractable Cu. As a result, further analyses were done using only total Cu contents. For air dried samples, separate regressions between WDC and total Cu concentration and water content yielded R^2 values of 0.81 and 0.62, respectively. For field moist samples, the R^2 values were 0.98 for both total Cu

Table 2
Effect of copper concentration on soil bulk density, total porosity, volumetric water content and void ratio (at −100 hPa matric potential).

Copper concentration ($mg\ kg^{-1}$)	Bulk density ($g\ cm^{-3}$)	Total porosity	Vol. water content ($m^3\ m^{-3}$)	Void ratio ($m^3\ m^{-3}$)
22 ± 1	1.19 ± 0.02[a]	0.54 ± 0.01[a]	0.27 ± 0.005[a]	1.19 ± 0.03[a]
48 ± 4	1.22 ± 0.03[ac]	0.53 ± 0.01[ac]	0.27 ± 0.006[a]	1.15 ± 0.06[ac]
175 ± 6	1.15 ± 0.04[a]	0.56 ± 0.01[a]	0.28 ± 0.006[a]	1.29 ± 0.07[a]
466 ± 18	1.34 ± 0.02[b]	0.48 ± 0.01[b]	0.27 ± 0.006[a]	0.94 ± 0.04[c]
875 ± 21	1.26 ± 0.03[abc]	0.51 ± 0.01[abc]	0.28 ± 0.005[a]	1.06 ± 0.05[abc]
2228 ± 83	1.27 ± 0.02[bc]	0.51 ± 0.01[bc]	0.33 ± 0.003[b]	1.04 ± 0.02[bc]
3837 ± 158	1.28 ± 0.01[c]	0.51 ± 0.01[c]	0.34 ± 0.004[b]	1.02 ± 0.02[b]

Mean values ± standard error ($n = 7$). Differently lettered means are significantly different at $p < 0.05$.

content and water content. Obviously, these two factors had a significant effect on WDC for both air dried and field moist samples. Multiple regressions using total Cu and water content, θ as regressors ($\mathrm{WDC} = a + b_1\theta + b_2\mathrm{Cu}$) yielded higher regression coefficients for both air dried (0.83) and field moist (0.99) samples. Total Cu and θ both significantly affected the WDC for both moisture contents, and the combined effect of the two variables explains 83% and 99%, of the variation of WDC for air-dried and field moist aggregates, respectively.

Soil air filled porosity, ε, air permeability, k_a, and void ratio all showed identical values for Cu levels up to 875 $mg\ kg^{-1}$ (Fig. 3; Table 2). When Cu concentration reached 2228 $mg\ kg^{-1}$ and higher, statistically significant lower values were observed (Fig. 3). The k_a, and its derivatives, soil pore organisation, PO_1 (k_a/ε) and PO_2 (k_a/ε^2) are useful for characterising soil pore geometry and hence soil structure (Blackwell et al., 1990; Groenevelt et al., 1984), with highly structured soils usually having greater k_a and PO values. The relationship between PO_1 and ε for the seven study areas is shown in Fig. 4. Isolines of k_a (10, 30 and 60 μm^2) are also added and emphasise the trends in k_a already shown in Fig. 3b. Generally, PO reflects the ability of a unit volume of air-filled pores to conduct gas by convection and is thus an indicator of the arrangement, shape and tortuosity of the macropore space. Groenevelt et al. (1984) stated that differences in PO_1 and PO_2 reflect differences in pore size distribution and continuity because any two soils with similar pore size distribution and continuity will always have the same PO_1 and PO_2 since any extra ε will contribute proportionally to k_a. The areas with Cu < 176 mg g^{-1} had PO_1 values above the $k_a = 60\ \mu m^2$ isoline, and the greatest Cu concentrations showed lowest PO_1. Additionally, similar trends observed for PO_2 (data not shown) with increasing copper content suggest that Cu contamination affects not just ε, but also the distribution and continuity of the pores. However, the question here is whether the decreasing k_a (and PO) from Cu > 466 $mg\ kg^{-1}$ is due to the direct effect of Cu or is merely a result of decreased ε. This is because k_a is highly dependent on ε (McCarthy and Brown, 1992; Moldrup et al., 1998). The k_a was linearly related to ε when average data (for each study area) was used (data not shown). The linear regression relationship obtained from k_a and ε ($k_a = 662\varepsilon - 98.9$; $R^2 = 0.95$) implies that at a ε value below 0.15 $m^3\ m^{-3}$, gas transport by convection ceases completely. It can be deduced from the above that increasing Cu concentrations led to decreased ε (smaller pore sizes with a higher degree of discontinuity due to lower earthworm and plant root activity) which resulted in lower k_a and PO values.

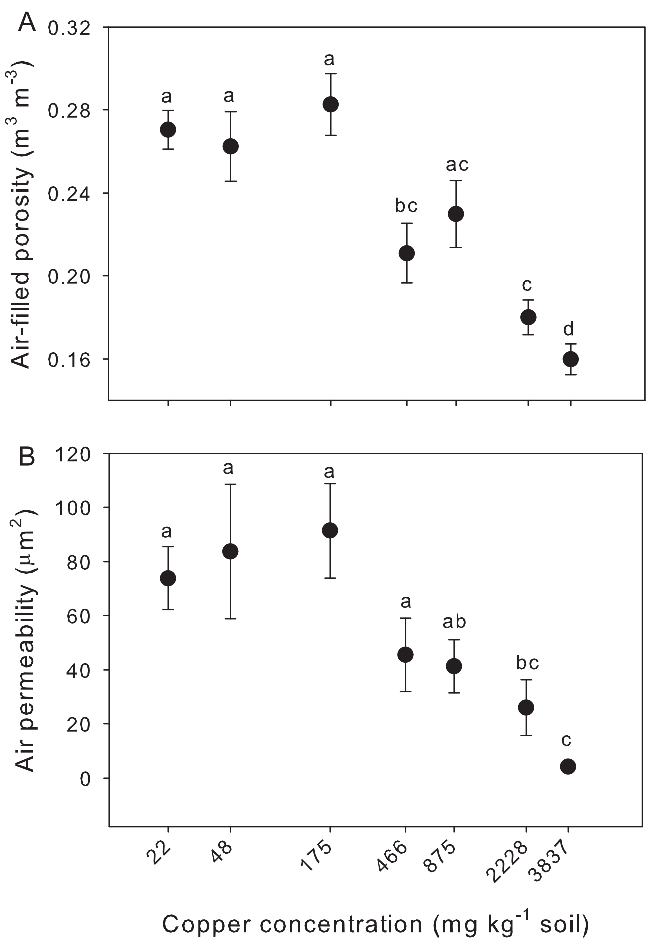

Fig. 3. Effect of copper concentration on (A) soil air-filled porosity and (B) soil air permeability at −100 hPa matric potential. Bars indicate SEM. Differently lettered means are significantly different at $p < 0.05$.

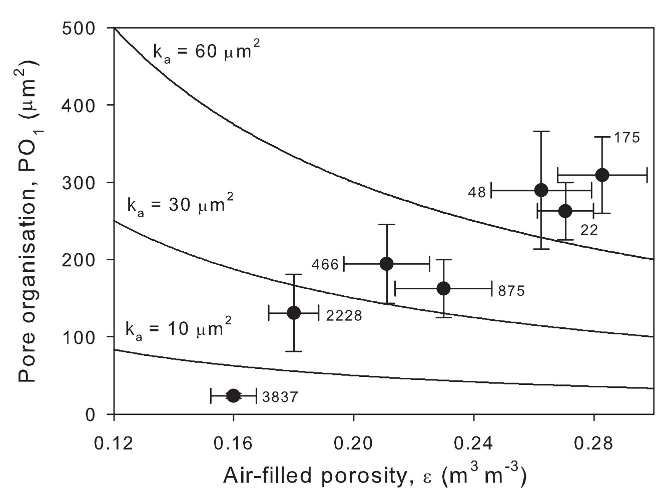

Fig. 4. Mean values of soil pore organisation, PO_1, (air permeability, k_a/air-filled porosity, ε) as a function of air filled porosity, ε, for seven copper concentration levels in $mg\ kg^{-1}$ (shown adjacent to data points). Air permeability, k_a isolines are shown. Bars indicate SEM.

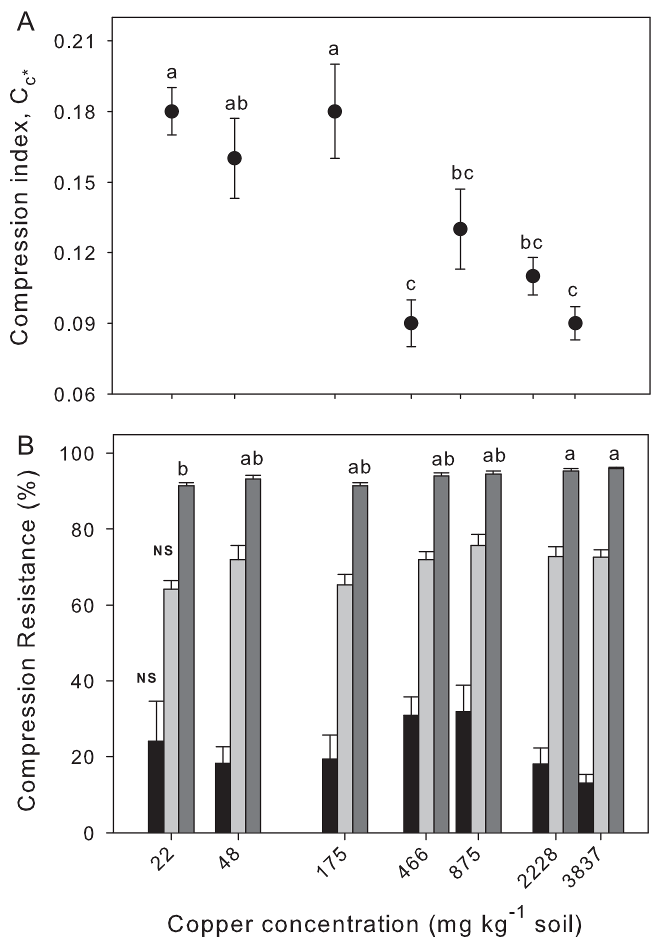

Fig. 5. (A) Compression index, C_{c^*} obtained from the Gompertz model as a function of copper concentration and (B) compression resistance indicated by relative changes in air permeability (■), air filled porosity (□) and void ratio (■) for different copper concentration levels. Bars indicate SEM. Differently lettered means are significantly different at $p < 0.05$. NS indicates no significant differences among the different concentration levels for that variable.

3.4. Resistance of soil structure to mechanical stress (compression)

The soil's resistance to mechanical stress was quantified using two methods. First, the general resistance (related to soil bulk density) was quantified by using the compression index, C_{c^*}, obtained using the Gompertz model (Eqs. (4) and (5)). Second, relative changes in k_a, ε and e following compression (Eq. (2)) were used as an indicator of the soil's resistance.

When using C_{c^*}, soils with smaller values are more resistant to compression and vice versa. The study areas with Cu contents up to 175 mg kg^{-1} showed a lower resistance to compression compared to the areas with Cu contents from 466 mg kg^{-1} and above (Fig. 5a). However, this lower general resistance to compression may not be a direct result of the Cu concentration but rather through the effect of Cu on soil bulk density/void ratio as explained above. Other factors affecting C_{c^*} are the water content; OM content and strength of structural units (Smith et al., 1997; Keller et al., 2011; Arthur et al., 2012). The C_{c^*} was positively correlated with the initial void ratio, e_0, ($R^2 = 0.90$; $p < 0.05$) and negatively correlated with water content ($R^2 = 0.29$; $p < 0.05$) in this study. The lower C_{c^*} at higher water contents coupled with lower e_0 may be due to pore water pressure which buffers the effect of the imposed stress. Also, the significant positive correlation between C_{c^*} and e_0 is because soils with higher e_0 have more pore space that can be removed compared to more compact soils. There was no significant correlation between C_{c^*} and OM as also shown by Zhang et al. (1997) possibly due to the confounding effect of Cu on the e_0 and water content (Table 1).

The relative changes in the three indicators following compression are shown in Fig. 5b. When looking at the relative changes, it is important to consider the initial state of the soils before compression. There was no significant difference in the changes in k_a and e for all seven study areas, although there was a trend of lower resistance (for k_a) for the two areas with the highest Cu content. This means that although there was a decreasing k_a and ε with increasing Cu content (Fig. 3), the reduction in the two parameters was similar after compression. When considering e, there was a significantly higher resistance for the two highest Cu content areas compared to the control. This is because contamination indirectly increased soil density, decreasing ε and reducing its susceptibility to compression. The general trend of e for all the study areas was expectedly similar to that of C_{c^*}, since C_{c^*} is strongly dependent on e. This also shows that the use of C_{c^*} as an indicator of compression resistance is accurate, even at low applied stresses (200 kPa in this case).

3.5. Resilience of soil structure to compression

The resilience of the soil for the different study areas was quantified as the relative recovery compared to the value after compression (Eq. (3)). In natural environments, wetting–drying and freezing–thawing cycles are essential mechanisms governing soil structure recovery. Soil properties such as soil total porosity (Pires et al., 2005), permeability and soil volume (Viklander, 1998) are positively affected by wet–dry and freeze–thaw cycles. As stated earlier, the recovery of structure should be understood in the context of the original state of the soil, as well as its resistance to the stress. Recovery of structure following wet–dry cycles arise from the expansion of the electrical double layer, differential swelling and slaking during saturation followed by differential settling of finer particles between coarser particles and particle re-orientation during drying (Shiel et al., 1988). In this study, after exposure to wet–dry cycles, there was a general trend of increasing recovery of k_a with increasing Cu content and increasing OM (Fig. 6). The areas with Cu ≥ 2228 mg kg^{-1} showed significantly higher resilience (>100%) increase compared to the rest. This could be partly because k_a was significantly lower for these areas at the beginning of the compression test. There was no clear trend for resilience using ε, although the areas with intermediate Cu content had lower recovery. Recovery of e was less than 1 for all the study areas, with no significant differences between them, possibly because the e was highly resistant to compression (Fig. 6).

Further, freeze–thaw cycles led to considerable recovery of k_a for all study areas. There was a trend of increasing recovery of k_a with increasing Cu content; however there was no significant difference between them. Increased recovery of k_a may be due to micro-fissuring during the cycles and the pores that remain after thawing of the ice crystals. Recovery of e and ε showed no clear trend and generally increased less than 1% and 10% from the compressed state, respectively. Comparatively, wet–dry cycles resulted in much higher recovery for all three parameters than freeze–thaw cycles as reported in recent studies (Gregory et al., 2009; Arthur et al., 2012).

3.6. Relations between microbial relations and soil structure

Linking microbial enzyme activity with soil structure is a necessary step to a holistic approach in assessing the impact of Cu contamination on the soil ecosystem. It is likely that the significant effect of Cu on WDC is through the effect of Cu on microbial

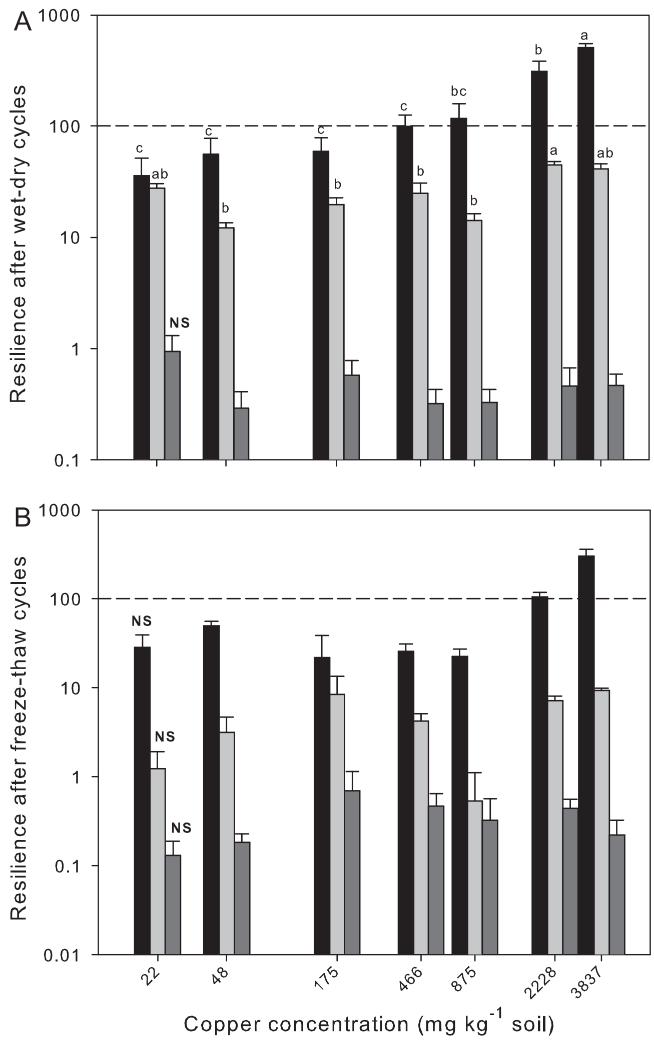

Fig. 6. Resilience after soil compression indicated by relative changes in air permeability (■), air filled porosity (□) and void ratio (■) for different copper concentration levels after (A) wet–dry and (B) freeze–thaw cycles. Bars indicate SEM. Differently lettered means are significantly different at $p < 0.05$. NS indicates no significant differences among the different concentration levels for that variable.

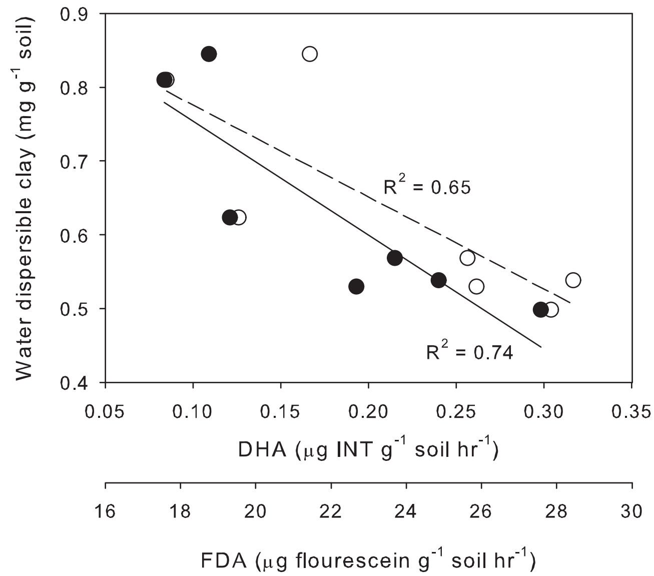

Fig. 7. Relationship between water dispersible clay and microbial activity quantified by dehydrogenase activity, DHA (●) and flourescein diacetate and FDA (○) for air dried samples.

activity (Fig. 1). Low microbial activity (especially for DHA) was associated with high WDC for air dried aggregates (Fig. 7). One likely reason for this is that soil fungi significantly contribute to the formation and stabilisation of microaggregates by connecting the soil particles with their hyphae and other components such as ergosterol (Elmholt et al., 2008). Another possible reason are microbial extracellular polymeric substances and patchy organic material from microbial cell envelopes (Miltner et al., 2011), which might also connect and thus stabilise mineral particles. Higher microbial enzyme activity will therefore mean more stable aggregates and lower amounts of WDC. In the broader perspective, when tillage operations ceased on the field, with no further external disturbances, the soil–microbe system of the various parts of the field (different contamination levels) would have self-organised based on the available substrates and conditions. This process spontaneously evolved the soil structure from the previous state to its present state out of an interaction between microbial activity, soil aggregation and flow of water and air in the soil (Crawford et al., 2011). Expectedly, areas with Cu < 466 mg kg^{-1} showed more proliferation of plant residues and roots, leading to higher micro- and macro-organism activity, further providing adequate aeration for plant roots culminating in more stable aggregates, improved permeability and higher complexity of the air-filled pore space (indicated by the higher pore organisation). On the other hand, the highly polluted areas will over time spontaneously develop a more compact, dense structure emanating from the decreased plant biomass and macro- and micro-organism activity. This dense structure facilitated increased resistance to mechanical stress. There may, however be limits to how far self-organisation can account for all observed differences over time in fallow fields. This will be looked at in future studies.

4. Conclusions

A history of copper pollution (with total concentrations as high as ~3800 mg kg^{-1} soil) on a field currently under fallow had significant negative impact on total microbial activity with an effect concentration causing a 50% reduction (EC_{50}) in enzyme activity observed at ~500 mg kg^{-1}. The reduced microbial activity in the polluted areas coupled with previously reported decreases in earthworm and plant populations resulted in a more compact structure (higher bulk density and lower air permeability and pore organisation) composed of aggregates with greater susceptible to dispersion by water. Copper pollution (Cu $\gtrsim$ 500 mg kg^{-1}) reduced the general susceptibility of the soil to laboratory-imposed compression stress due to its indirect increasing effect on soil density. Both resistance and resilience to stress application were positively affected by Cu pollution, due to the denser structure of the polluted soils. In perspective, total Cu concentrations of ~500 mg kg^{-1} can serve as a tentative threshold concentration beyond which soil microbial activity and structural integrity are affected negatively. We note that this copper threshold may apply only to long-term polluted fields with similar soil texture, and may have to be tested against freshly polluted fields and for soils with different levels of clay and organic matter. Since elevated copper levels were found to markedly affect a number of basic biological, physical, and mechanical properties, there is likely an impact also on essential soil ecosystem services such as the ability to transport and clean infiltrating water, the transport and fate of oxygen and climate gases, and the functional soil architecture and its physico-chemical life support systems (de Jonge et al., 2009; Robinson et al., 2012). On-going investigations at the Hygum site will attempt to further address these issues.

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References

Arthur, E., Schjønning, P., Moldrup, P., de Jonge, L.W., 2012. Soil resistance and resilience to mechanical stresses for three differently managed sandy loam soils. Geoderma 173–174, 50–60.
Bååth, E., 1989. Effects of heavy-metals in soil on microbial processes and populations (a review). Water Air Soil Pollut. 47, 335–379.
Blackwell, P.S., Ringrosevoase, A.J., Jayawardane, N.S., Olsson, K.A., Mckenzie, D.C., Mason, W.K., 1990. The use of air-filled porosity and intrinsic permeability to air to characterize structure of macropore space and saturated hydraulic conductivity of clay soils. J. Soil Sci. 41, 215–228.
Brandt, K.K., Frandsen, R.J.N., Holm, P.E., Nybroe, O., 2010. Development of pollution-induced community tolerance is linked to structural and functional resilience of a soil bacterial community following a five-year field exposure to copper. Soil Biol. Biochem. 42, 748–757.
Burns, R.G., 1982. Enzyme-activity in soil – location and a possible role in microbial ecology. Soil Biol. Biochem. 14, 423–427.
Chander, K., Brookes, P.C., 1991. Is the dehydrogenase assay invalid as a method to estimate microbial activity in copper-contaminated soils? Soil Biol. Biochem. 23, 909–915.
Chaperon, S., Sauvé, S., 2008. Toxicity interactions of cadmium, copper, and lead on soil urease and dehydrogenase activity in relation to chemical speciation. Ecotoxicol. Environ. Saf. 70, 1–9.
Crawford, J.W., Deacon, L., Grinev, D., Harris, J.A., Ritz, K., Singh, B.K., Young, I., 2011. Microbial diversity affects self-organization of the soil–microbe system with consequences for function. J. R. Soc. Interface 9, 1302–1310, http://dx.doi.org/10.1098/rsif.2011.0679.
Czyz, E.A., Dexter, A.R., Terelak, H., 2002. Content of readily dispersible clay in the arable layer of some polish soils. In: Pagliai, M., Jones, J. (Eds.), Sustainable Land Management – Environmental Protection – A Soil Physical Approach. Advances in Geoecology. Catena Verlag, Germany, pp. 115–124.
de Jonge, L.W., Moldrup, P., Schjonning, P., 2009. Soil infrastructure interfaces & translocation processes in inner space ('Soil-it-is'): towards a road map for the constraints and crossroads of soil architecture and biophysical processes. Hydrol. Earth Syst. Sci. 13, 1485–1502.
Dexter, A.R., 1988. Advances in characterization of soil structure. Soil Till. Res. 11, 199–238.
Dexter, A.R., Richard, G., Czyz, E.A., Davy, J., Hardy, M., Duval, O., 2011. Clay dispersion from soil as a function of antecedent water potential. Soil Sci. Soc. Am. J. 75, 444–455.
Didden, W.A.M., 1990. Involvement of Enchytraeidae (Oligochaeta) in soil structure evolution in agricultural fields. Biol. Fertil. Soils 9, 152–158.
Ekwue, E.I., 1990. Organic-matter effects on soil strength properties. Soil Till. Res. 16, 289–297.
Elmholt, S., Schjønning, P., Munkholm, L.J., Debosz, K., 2008. Soil management effects on aggregate stability and biological binding. Geoderma 144, 455–467.
Fernández-Calviño, D., Martin, A., Arias-Estevez, M., Baath, E., Diaz-Ravina, M., 2010. Microbial community structure of vineyard soils with different pH and copper content. Appl. Soil Ecol. 46, 276–282.
Flint, A.L., Flint, L.E., 2002a. Particle density (2.3.2.2., liquid displacement). In: Dane, J.H., Topp, G.C. (Eds.), Methods of Soil Analysis: Part 4. Physical Methods. SSSA, Madison, WI, pp. 230–232.
Flint, A.L., Flint, L.E., 2002b. Porosity (2.3.2.1., calculation from particle and bulk densities). In: Dane, J.H., Topp, G.C. (Eds.), Methods of Soil Analysis: Part 4. Physical Methods. SSSA, Madison, WI, pp. 241–254.
García, C., Hernández, T., Costa, F., Ceccanti, B., Masciandaro, G., 1993. The dehydrogenase activity of soil as an ecological marker in processes of perturbed system regeneration. In: Gallardo-Lancho, J. (Ed.), Proceeding of the XI International Symposium of Environmental Biochemistry. Salamanca, Spain, pp. 89–100.
Gee, G.W., Bauder, J.W., 1986. Particle size analysis. In: Klute, A. (Ed.), Methods of soil analysis: Part 1 Agron. Monogr, vol. 9, second ed. ASA and SSSA, Madison, WI, pp. 383–423.
Giller, K.E., Witter, E., McGrath, S.P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30, 1389–1414.
Gompertz, B., 1825. On the nature of the function expressive of the law of human mortality, and on a new model of determining the value of life contingencies. Philos. Trans. R. Soc. Lond. 115, 513–585.
Gregory, A.S., Watts, C.W., Griffiths, B.S., Hallett, P.D., Kuan, H.L., Whitmore, A.P., 2009. The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. Geoderma 153, 172–185.
Gregory, A.S., Whalley, W.R., Watts, C.W., Bird, N.R.A., Hallett, P.D., Whitmore, A.P., 2006. Calculation of the compression index and precompression stress from soil compression test data. Soil Till. Res. 89, 45–57.
Groenevelt, P.H., Kay, B.D., Grant, C.D., 1984. Physical assessment of a soil with respect to rooting potential. Geoderma 34, 101–114.
Holmstrup, M., Hornum, H.D., 2012. Earthworm colonisation of abandoned arable soil polluted by copper. Pedobiologia 55, 63–65.
Iversen, B.V., Schjonning, P., Poulsen, T.G., Moldrup, P., 2001. In-situ, on-site and laboratory measurements of soil air permeability: boundary conditions and measurement scale. Soil Sci. 166, 97–106.
Kay, B.D., Dexter, A.R., 1992. The influence of dispersible clay and wetting drying cycles on the tensile-strength of a red-brown earth. Aust. J. Soil Res. 30, 297–310.
Keller, T., Lamande, M., Schjønning, P., Dexter, A.R., 2011. Analysis of soil compression curves from uniaxial confined compression tests. Geoderma 163, 13–23.
Kizilkaya, R., Askin, T., Bayrakli, B., Saglam, N., 2004. Microbiological characteristics of soils contaminated with heavy metals. Eur. J. Soil Bio. 40, 95–102.
Kjaergaard, C., de Jonge, L.W., Moldrup, P., Schjønning, P., 2004. Water-dispersible colloids: effects of measurement method, clay content, initial soil matric potential, and wetting rate. Vadose Zone J. 3, 403–412.
Koolen, A.J., 1974. A method for soil compactibility determination. J. Agric. Eng. Res. 19, 271–278.
Lamandé, M., Labouriau, R., Holmstrup, M., Torp, S.B., Greve, M.H., Heckrath, G., Iversen, B.V., de Jonge, L.W., Moldrup, P., Jacobsen, O.H., 2011. Density of macropores as related to soil and earthworm community parameters in cultivated grasslands. Geoderma 162, 319–326.
Lamandé, M., Schjønning, P., 2008. The ability of agricultural tyres to distribute the wheel load at the soil-tyre interface. J. Terramechanics 45, 109–120.
Lee, K., 1985. Earthworms. Their Ecology and Relationships With Soils and Land Use. Academic Press, Sydney.
Ma, Y.B., Lombi, E., Oliver, I.W., Nolan, A.L., McLaughlin, M.J., 2006. Long-term aging of copper added to soils. Environ. Sci. Technol. 40, 6310–6317.
Maraldo, K., Christensen, B., Strandberg, B., Holmstrup, M., 2006. Effects of copper on enchytraeids in the field under differing soil moisture regimes. Environ. Toxicol. Chem. 25, 604–612.
McCarthy, K.P., Brown, K.W., 1992. Soil gas-permeability as influenced by soil gas-filled porosity. Soil Sci. Soc. Am. J. 56, 997–1003.
Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2011. SOM genesis: microbial biomass as a significant source. Biogeochemistry 22, 139–143.
Moldrup, P., Olesen, T., Komatsu, T., Schjønning, P., Rolston, D.E., 2001. Tortuosity, diffusivity, and permeability in the soil liquid and gaseous phases. Soil Sci. Soc. Am. J. 65, 613–623.
Moldrup, P., Poulsen, T.G., Schjonning, P., Olesen, T., Yamaguchi, T., 1998. Gas permeability in undisturbed soils: measurements and predictive models. Soil Sci. 163, 180–189.
Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon and organic matter. In: Sparks, D.L., et al. (Eds.), Methods of Soil Analysis. Part 3, Chemical Methods. Soil Science Society of America Book Series No. 5. ASA and SSSA, Madison, WI, pp. 961–1010.
Novozamsky, I., Lexmond, Th.M., Houba, V.J.G., 1993. A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Int. J. Environ. Anal. Chem. 51, 47–58.
Oades, J.M., 1984. Soil organic-matter and structural stability – mechanisms and implications for management. Plant Soil 76, 319–337.
Obbard, J.P., 2001. Measurement of dehydrogenase activity using 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) in the presence of copper. Biol. Fertil. Soils 33, 328–330.
Page, A.L., Miller, R.H., Keeney, D.R., 1982. Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties, vol. 9., second ed. ASA, Madison, WI, pp. 149–157.
Pedersen, M.B., Axelsen, J.A., Strandberg, B., Jensen, J., Attrill, M.J., 1999. The impact of a copper gradient on a microarthropod field community. Ecotoxicology 8, 467–483.
Pedersen, M.B., van Gestel, C.A.M., 2001. Toxicity of copper to the collembolan Folsomia fimetaria in relation to the age of soil contamination. Ecotoxicol. Environ. Saf. 49, 54–59.
Pires, L.F., Bacchi, O.O.S., Reichardt, K., 2005. Gamma ray computed tomography to evaluate wetting/drying soil structure changes. Nucl. Instrum. Methods B 229, 443–456.
Reid, J.B., Goss, M.J., 1981. Effect of living roots of different plant species on the aggregate stability of two arable soils. J. Soil Sci. 32, 521–541.
Robinson, D.A., Hockley, N., Dominati, E., et al., 2012. Natural capital, ecosystem services, and soil change: why soil science must embrace an ecosystems approach. Vadose Zone J. 11 (1), http://dx.doi.org/10.2136/vzj2011.0051.
Sauve, S., 2006. Copper inhibition of soil organic matter decomposition in a seventy-year field exposure. Environ. Toxicol. Chem. 25, 854–857.
Schjønning, P., Elmholt, S., Munkholm, L.J., Debosz, K., 2002. Soil quality aspects of humid sandy loams as influenced by organic and conventional long-term management. Agric. Ecosyst. Environ. 88, 195–214.
Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol. 43, 1256–1261.
Scott-Fordsmand, J.J., Krogh, P.H., Weeks, J.M., 2000. Responses of Folsomia fimetaria (Collembola: Isotomidae) to copper under different soil copper contamination histories in relation to risk assessment. Environ. Toxicol. Chem. 19, 1297–1303.
Shiel, R.S., Adey, M.A., Lodder, M., 1988. The effect of successive wet dry cycles on aggregate size distribution in a clay texture soil. Journal of Soil Science 39, 71–80.

Six, J., Elliott, E.T., Paustian, K., 2000. Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy. Soil Sci. Soc. Am. J. 64, 1042–1049.
Smith, C.W., Johnston, M.A., Lorentz, S., 1997. Assessing the compaction susceptibility of south african forestry soils.1 The effect of soil type, water content and applied pressure on uni-axial compaction. Soil Till. Res. 41, 53–73.
Spurgeon, D.J., Hopkin, S.P., 1995. Extrapolation of the laboratory based OECD earthworm toxicity test to metal contaminated field sites. Ecotoxicology 4, 190–205.
Strandberg, B., Axelsen, J.A., Pedersen, M.B., Jensen, J., Attrill, M.J., 2006. Effect of a copper gradient on plant community structure. Environ. Toxicol. Chem. 25, 743–753.
Tom-Petersen, A., Hansen, H.C.B., Nybroe, O., 2004. Time and moisture effects on total and bioavailable copper in soil water extracts. J Environ. Qual. 33, 505–512.
Vendelboe, A.L., Moldrup, P., Schjønning, P., Oyedele, D.J., Jin, Y., Scow, K., de Jonge, L.W., 2012. Colloid release from soil aggregates: application of laser diffraction. Vadoze Zone J., http://dx.doi.org/10.2136/vzj2011.0070.
Viklander, P., 1998. Permeability and volume changes in till due to cyclic freeze/thaw. Can. Geotech. J. 35, 471–477.
Xie, W.J., Zhou, J.M., Wang, H.Y., Chen, X.Q., Lu, Z.H., Yu, J.B., Chen, X.B., 2009. Short-term effects of copper, cadmium and cypermethrin on dehydrogenase activity and microbial functional diversity in soils after long-term mineral or organic fertilization. Agric. Ecosyst. Environ. 129, 450–456.
Zhang, H.Q., Hartge, K.H., Ringe, H., 1997. Effectiveness of organic matter incorporation in reducing soil compactibility. Soil Sci. Soc. Am. J. 61, 239–245.