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Long-term impacts of zinc and copper enriched sewage sludge additions on bacterial, archaeal and fungal communities in arable and grassland soils

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ABSTRACT

Long-term impacts of metal contamination derived from sewage sludge on soil microbial communities have been widely evaluated, but confounding effects have made it difficult to draw firm conclusions and thus to advise on safe metal limits. Here we used Multiplex-terminal restriction length fragment polymorphism (M-TRFLP) to assess the long-term impact of sludge-borne Zn and Cu contamination on the structure of bacterial, fungal and archaeal communities across seven different soils at metal levels relevant to current guideline limits. Despite strong effects of site on microbial community structure, analysis of similarity (ANOSIM) demonstrated a small but significant effect of Zn on bacteria ($P < 0.001$), archaea ($P < 0.001$), and fungi ($P < 0.001$). Significant effects of Cu on bacteria ($P < 0.001$), archaea ($P < 0.001$) and fungi ($P < 0.001$) were also observed. Several bacterial and fungal T-RFs were identified as responding to Zn or Cu. For example the bacterial T-RF 72 was negatively correlated with Zn and Cu, and T-RF 259 was positively correlated with Zn. Attempts to identify these bacterial markers of Zn and Cu contamination suggest a negative impact of Cu on Acidobacteria in arable soils. These results demonstrate for the first time, that despite a strong influence of site on microbial community structure, effects of Zn and Cu derived from sewage sludge can be detected as shifts in bacterial, fungal and archaeal communities indicating a common response more than 11 years after sludge addition.

1. Introduction

Land application of sewage sludge is an attractive recycling approach because of the benefits to plant growth and soil quality. However, sewage sludge also contains contaminants that may affect soil microbial communities and their processes that are fundamental to maintaining soil health and ecosystem function. Some metals, including Zn and Cu, are of particular concern because of their persistence and potential toxicity in soils. While in general, the impacts of potentially toxic metals on soil microbial communities and processes has been well studied (see Giller et al., 1998 for review), we are still unable to draw firm conclusions as to the long-term effects of these metals on soil microbial communities. This is in part due to the variation in the microbial communities' response to these metals across studies (Anderson et al., 2009), as well as the presence of multiple metals in some studies (e.g. Sandaa et al., 1999; Sandaa et al., 2001). This, together with a continued lack of understanding as to how micro-organisms are exposed to metals in soil (Giller et al., 2009), makes it difficult to determine which metals are having a negative impact. With respect to contaminated sludge application to land, interpretation of results is often confounded by the short-term effects of organic additions (Speir et al., 2007). Over the short term, sludge application undoubtedly improves the soil physical (e.g. Sort and Alcaniz, 1999; Griffiths et al., 2005) and chemical (e.g. Speir et al., 2004) status and generally promotes microbial growth and activity (Debosz et al., 2002; Garcıa-Gil et al., 2004). This may mask any negative effects brought about by the presence of contaminants. Over the long-term however, the accumulation of potentially toxic metals through repeated sludge additions could have a detrimental influence on soil microbial communities and their functions and thus threaten the long-term viability of sludge application to land. Reported long-term effects of sludge on the soil microbial biomass are varied (Brookes and McGrath, 1984; Flieβach et al., 1994; Chander et al., 1995; Defra, 2005; Reneall et al., 2007; Speir et al., 2007) and although such measurements

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can provide information concerning the microbial biomass as a whole, it provides little information on community composition. Additionally, whether microbial biomass is a sensitive ecotoxicological endpoint at the field scale has recently been questioned (Broos et al., 2007).

Microbial community composition is an important consideration, as changes may alter the physiological and functional capacity of the community (Waldrop et al., 2000) which could be reflected in nutrient cycling and ecosystem functioning. Persistent metal effects on soil microbial community structure under field conditions have previously been demonstrated using a range of community profiling approaches (Moffett et al., 2003; Abaye et al., 2005; Macdonald et al., 2007, 2008). Terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu et al., 1997) is widely used to assess changes in microbial community structure by comparing the gain, loss or change in the abundance of specific fragments within a community across temporal and spatial scales, or following a perturbation (Schütte et al., 2008). This method, although sensitive to the inherent biases of any polymerase chain reaction (PCR)-based approach, provides a rapid and reproducible assessment of changes in community composition. When combined with cloning and sequencing, or used in conjunction with *in silico* digestion, T-RFLP analysis may provide better characterisation of microbial communities within complex environmental samples (Junier and Witzel, 2008). It has been suggested that T-RFLP could be used to identify markers of long-term metal effects in soil (Macdonald et al., 2008). This may have application for monitoring purposes. However, it remains to be seen whether changes in microbial community composition in response to long-term additions of metal-contaminated sludge are consistent across different soil types and geographic locations.

A series of experimental plots were implemented in 1994 to test the sustainability of current European Union (EU) soil metal limits (Gibbs et al., 2006). These experiments present a unique opportunity to assess the impacts of metal-rich sludge additions across a range of soil types and land uses. Here we evaluate the long-term impacts of Zn- and of Cu-contaminated sludge on bacterial, archaeal and fungal community structure across five arable soils and two grassland soils: (1) to determine if microbial communities respond to Zn or Cu similarly across sites, and (2) to identify the potential microbial indicators of change that could be used for monitoring purposes.

2. Materials and methods

2.1. Experimental sites

Seven experimental field sites were used in the study and are part of a larger study encompassing nine experimental field sites throughout the UK (Gibbs et al., 2006). Of the seven sites evaluated here, five were under arable management and two under grassland management. These corresponded to a range of soil texture and organic matter contents. Basic soil properties, treatments and metal concentrations are given in Table 1. Across all sites, individual metal (Zn or Cu) dose response treatments were established between 1994 and 1997 by annual additions of sewage sludges predominantly enriched with either Zn or Cu. A key feature of the experiments is that the same total amount of sewage sludge organic matter was added and the same sludges used at all sites (Gibbs et al., 2006; Chaudri et al., 2008). Briefly, nine treatments were imposed. The first set of treatments consisted of: no sludge, uncontaminated digested sludge and three Zn rich sludge treatments aimed at 250, 350 and 450 mg Zn kg^{-1} soil. The second set of treatments consisted of: no sludge, uncontaminated undigested sludge and three Cu rich sludge treatments aimed at 100, 150 and 200 mg Cu kg^{-1} soil. Actual total soil Zn and Cu concentrations for each site are listed in Table 1. The same no sludge control plot was used for both the Zn and Cu treatments. Each treatment was in triplicate in a randomized block design. Prior to sampling in May 2008, the soils had not received sludge additions since 1997. For the arable sites, plots had been under wheat cultivation since 2005, prior to which they had been under wheat/ryegrass rotation since 1997. The grassland sites had been under ryegrass and periodically re-sown since 1997.

2.2. Soil sampling and chemical analysis

Soils were sampled in May 2008, 11 years after the final sludge application. Fifteen to twenty soil cores (1.5 cm in diameter, 15 cm depth) were collected from each plot. Cores from within a plot were bulked, sieved (<2 mm) and split into three sub-samples that were either stored at 4 °C, air-dried for chemical analysis, or stored at −20 °C for molecular analyses. A sub-sample of the air-dried soil was ground (<0.5 mm) and used for determination of total Zn, Cu, Al, Ca, Fe, P and S using aqua regia digestion (McGrath and Cunliffe, 1985). Results of the concentrations of metals extractable in 1 M

Table 1
Basic soil properties and mean treatment top soil metal concentrations (mg kg^{-1}) for Zinc and Copper amended soils. Values in brackets are ± SE, $n = 3$.

	Arable					Grassland	
	Gleadthorpe	Woburn	Watlington	Rosemaund	Bridgets	Auchincruive	Hartwood
Soil Type[a] and pH[b]	Cambisol (7.1)	Arenosol (7.2)	Cambisol (7.4)	Luvisol (7.0)	Rendzina (6.8)	Fluvisol (6.0)	Cambisol (5.8)
% Silt/Clay/Sand[b]	71/22/7	80/12/8	50/32/18	8/67/25	10/60/30	51/29/20	59/20/21
% Org C[b]	1.2	1.3	1.3	1.7	1.5	2.5	4.7
Treatments				Zn (mg kg^{-1})			
No Sludge	46 (0.72)	66 (2.92)	47 (1.05)	75 (1.43)	51 (0.67)	73 (0.32)	86 (1.28)
DS Control	82 (8.51)	94 (6.26)	83 (2.67)	90 (1.19)	74 (6.00)	107 (1.58)	107 (4.71)
DS Zn 250	229 (28.73)	235 (11.05)	252 (6.18)	201 (4.99)	171 (11.24)	335 (9.68)	306 (4.07)
DS Zn 350	323 (16.39)	306 (7.79)	330 (14.86)	261 (5.11)	254 (10.12)	396 (16.41)	395 (1.40)
DS Zn 450	326 (3.24)	421 (13.18)	466 (12.36)	346 (6.85)	355 (16.22)	552 (2.07)	540 (8.63)
				Cu (mg kg^{-1})			
No Sludge	11 (0.52)	17 (1.57)	11 (0.65)	18 (0.47)	11 (0.75)	20 (0.20)	29 (1.70)
UDS Control	32 (3.14)	36 (1.76)	37 (0.68)	33 (0.36)	35 (2.02)	557 (1.61)	56 (4.70)
UDS Cu 100	124 (12.17)	110 (1.92)	130 (3.58)	91 (2.21)	89 (2.77)	141 (1.29)	159 (2.75)
UDS Cu 150	155 (16.58)	164 (4.09)	181 (6.19)	131 (9.34)	130 (7.27)	165 (28.07)	216 (4.63)
UDS Cu 250	180 (13.01)	196 (8.04)	216 (3.82)	163 (3.39)	155 (6.04)	328 (5.12)	271 (6.11)

[a] FAO Soil Class (FAO, 1998).
[b] Mean soil pH from Gibbs et al. (2006). DS is Digested sludge. UDS is Undigested sludge.