

Structural and functional response of soil microbiota to addition of plant substrate are moderated by soil Cu levels

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Abstract In soils, the microbially mediated decomposition of plant residue is a key process with wide ranging effects on ecosystem functioning and stability. Understanding the impact of contamination on this process is of high importance. We investigated the effects of long-term (6 years) copper exposure on the capacity of soil microbiota to decompose newly added resources; dried and ground *Medicago truncatula* stubble. In addition, the effects on the microbial community structure across the three domains were explored using polymerase chain reaction–denaturing gradient gel electrophoresis rRNA gene profiling. Ecological distances in community structure between treatments was calculated (Kulczynski) and effects tested using PERMANOVA. Clear dose–response relationships were present between microbial respiration (CO₂ evolution) and soil Cu level in soils receiving medic, but

not under basal conditions (i.e., no medic added). These show that relatively labile forms of C are needed to drive microbial ecotoxicological responses and that microbial adaptation to the presence of Cu in the soils—after >6 years exposure—was functionally limited. Bacterial, archaeal and fungal communities showed significant ($P < 0.05$) levels of structural change in soils across the Cu gradient, demonstrating that species replacement had occurred following strong selective pressure. Addition of medic resources to the soils caused significant shifts in the bacterial and archaeal community structure ($P < 0.001$), which occurred across the entire range of soil Cu levels. For the fungal community, a significant interaction effect was present between Cu and medic addition ($P = 0.002$). At low Cu levels, medic addition caused large shifts in community structure, but this was

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negligible under high Cu levels. This was reflected in significant changes in the level of community structural dispersion at low compared with high Cu levels. As such, we show that Cu limits the capacity of soil fungal communities to rapidly respond to new resource capture. Given the primary role of soil fungi in plant material decomposition, this may have wide ranging impacts on wider ecosystem processes including nutrient cycling, trophic interactions, food web stability and energy transfer.

Keywords Decomposition · Microbial community structure · Copper

Introduction

The decomposition and recycling of organic matter is of fundamental importance to the functioning and stability of soil (and other) ecosystems (Swift et al. 1979). Organic matter decomposition underpins soil C-cycling, is intimately linked to the availability of other nutrients such as N and P (van Elsas et al. 2007) and provides energy for most other soil microbial processes that maintain ecosystem stability and function (e.g., aggregate formation, disease suppression via competitive exclusion).

The interactions between soil microorganisms and organic matter are complex. In particular, the quality of plant substrate (C:N ratio, extent of lignification, presence of secondary molecules) directly influences rate of degradation and proportion of plant-held C partitioned between the short- to mid-term available and recalcitrant fractions (Baldock et al. 1997). Organic matter entering soil ecosystems has direct impacts on soil microbial community structure and soil fauna (Wardle et al. 2006). Directly, this occurs via the selective effect of the resource (quality and quantity) on the decomposer community. For example, the high C:N ratio of plant material favour fungi over bacteria in early consumer–resource interactions. Indirectly, the addition of organic matter affects wider community organisation, for example by predator–prey and other trophic interactions extending from alteration of the decomposer community and coupled food webs (Moore et al. 2004). Thus, organic matter plays a central role in community organisation and the transfer and compartmentalisation of energy (Moore et al. 2004; Srivastava et al. 2009).

Factors affecting the structure of soil microbial communities may have effects on the ability of ecosystems to function. Such impacts can come from a variety of sources, including anthropogenic contamination by chemicals (pesticides, etc.), nutrient enrichment, metals and so forth. With increasing levels of contamination, the toxicological effect of such contaminants increases (e.g., Giller et al. 1998; Broos et al. 2005) sometimes after hormesis-type responses. When

impacting on primary ecosystem services, such as organic matter decomposition, the effects of soil contamination can have wide ranging impacts. Underpinning the functional stability of the soil ecosystem are microbial resistance and resilience mechanisms—i.e., the ability of the community to resist and recover from the effects of perturbation (respectively). These interrelated components of stability can be controlled by the soil microbial community and physiology as affected through changes in soil physicochemical properties (Griffiths et al. 2008).

We explored the links between increasing soil copper on microbial community assemblage and decomposition activity. The soil Cu gradient was one that had been established in an agricultural field in April 2002 (Broos et al. 2007), thus providing nearly 6 years in which adaptation or development of metal tolerance by the microbial community could occur prior to use of the soil in this study. A number of fundamental questions were posed. Firstly, how is decomposition of organic matter affected by long-term soil Cu exposure in the field (~6 years)? Are there differences in basal and substrate-induced respiration, or do responses only occur with addition of new, relatively labile C? What is the response of soil microbial community structure over the three domains (i.e., bacteria, fungi and archaea) to addition of a plant material? Finally, and most importantly, how does increasing levels of soil Cu affect the capacity of the soil microbial community to rapidly respond to addition of new energy resources?

Materials and methods

Soils sampling and processing

Soil was collected from a field trial site located on a grain cropping farm at Spalding, South Australia (approximately 160 km North of Adelaide). The soil pH is 6.3 (0.01M CaCl₂), CEC 18 cmol_c kg⁻¹, organic C and clay content 1.9% and 24%, respectively (Broos et al. 2007). Copper, as CuSO₄, had been applied to the field trial in 2002 across 12 application rates (details in Broos et al. 2007). Each treatment rate was applied to replicate field plots (3 m×4 m). For this study, samples (dry) were collected from the trial site in November 2007—i.e., ~6 years after establishment. Dry soil samples were sieved to 2 mm and stored at 4°C for 5 months until use. Equal volumes of soil from the replicate field plots were homogeneously combined into a single representative sample for each Cu rate. The total Cu (Cu_{Tot}) of these soils had been determined previously (Wakelin et al. 2009). By taking the average Cu_{Tot} value from the replicate samples, the composite average Cu_{Tot} were calculated as 56, 78, 82, 177, 213, 319, 528, 565, 1,065, 1,403, 3,094 and 3,225 mg Cu kg⁻¹ soil (hereon referred to as Cu 0 to Cu 11).

Soil microcosm incubation experiment

Soils for each of the 12 Cu levels were incubated with and without addition of dried, ground, barrel medic (*Medicago truncatula* c.v. Paraggio) material to provide basal and substrate-added treatments. Medic was chosen for the incubation experiments as the low C:N ratio of the plant material (C=39.8%, N=4.09%, oven dry basis) would ensure that C mineralisation was not N limited. In soil receiving medic, 3 wt.% of finely ground (<5 mm) plant material, along with 50 g soil, was added into a ‘zip-lock’ sealable plastic bag and the sample thoroughly mixed by shaking. For each sample, 18 g of soil was weighed into incubation pots that consisted of 50 mm high×37 mm i.d. sections of PVC pipelined with a fine nylon mesh at the base (0.75 µm; Australian Filter Specialists). Soil in each pot was pressed with a modified bottle-capping press to achieve a uniform bulk density of 1.5 g cm⁻³. The soils were adjusted to 60% WHC and placed into 1 L glass bottles (Bell® Quart Wide Mouth Mason Jars, Jarden Corporation). The jars were sealed with rubber-lined metal discs that had been modified to hold a septum to enable sampling of headspace gas. To maintain headspace humidity, small polycarbonate vials containing 10 mL of dH₂O were placed in each jar. The microcosms were incubated at 25°C for 20 days in the dark. Soil was collected at the end of the experiment for microbial community structure and community functional profiling. Regular samples of headspace gas were collected for CO₂ determination.

Soil respiration

Soil respiration was measured 12 times over a 9-day incubation period (0, 21, 27, 51, 69, 74, 96, 120, 143, 165, 187 and 211 h). For each sampling, headspace CO₂ in each microcosm was collected through the septum and analysed using a Servomex 1450 infrared gas analyser with sampling needle. The CO₂ (percentage) in each microcosm headspace was determined by comparing the output mV data against a CO₂ standard curve (0, 5, 10, 15, 25 and 50 mL CO₂ l⁻¹) which was freshly generated for each sampling time. When headspace CO₂ levels were projected to exceed the linear range of the instrument before the next sampling point, all microcosms were refreshed with atmospheric air, resealed, and the background level (with respect to the next sampling point) of CO₂ determined. The final CO₂ (percentage) headspace data was converted into micromole CO₂ g⁻¹ soil with respect to time.

For each time point, dose–response curves were used to fit soil Cu (log transformed) data with microbial respiration using the least-squares method. EC₅₀ values, i.e., the concentration of Cu which caused a 50% reduction in respiration, were calculated along with 95% confidence intervals.

The behaviour of respiration curves over time for different Cu doses was tested using a multivariate approach, allowing fully for the ‘repeated measures’ inherent in recording respiration rates from the same microcosm unit through time. For the basal and medic-added data sets, Manhattan distances were calculated between every pair of respiration curves (each from a different Cu level) providing, in effect, area between every pair of curves. The basal and medic-added distance matrices were separately tested for evidence of seriation (a trend across increasing Cu values) using a RELATE permutation test (Clarke et al. 1993), as implemented in the PRIMER software used for all the multivariate statistical computation of this paper (Clarke 1993; Clarke and Gorley 2006).

Microbial community fingerprinting

The bacterial, archaeal and fungal communities were characterised by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE). For the bacterial community, a variable region of the 16S rRNA gene was PCR-amplified with primers F986 and R1401 (Duineveld et al. 1998). For the soil fungal community, the ITS1–5.8S–ITS2 gene region (hereafter referred to as the ITS gene), was amplified with primers ITS1 and ITS2* (Gardes and Bruns 1993; Wakelin et al. 2007). The PCR and DGGE of the bacterial and fungal communities followed conditions described previously (Wakelin et al. 2009).

Archaeal community profiling was based on the nested PCR method of Øvreås et al. (1997). Primers PRA46f and PREA1100r were used in first-round PCR at 0.5 µM each; the PCR mix also contained 1 U of Qiagen HotStar Taq polymerase, 2.5 µL of 10×buffer, 10 mM of each dNTP, 4 µL DNA and water to 25 µL. First-round PCR was conducted using 30 cycles of denaturation at 92°C for 30 s, annealing at 53°C and extension at 72°C for 1 min each. Second-round PCR used primers PARCH340f and PARCH519r and other chemistry as before. Template consisted of 5 µL of 1:100-diluted first-round PCR product. PCR cycling was similar to before except primer annealing was conducted at 54°C. DGGE was conducted in a 6% acrylamide:bis-acrylamide (37.5:1) gel over a 40–60% formamide:urea denaturing range. Electrophoresis occurred at 60°C with 110 V for 17 h duration. Post-electrophoresis gel staining, image capture, etc. followed previously described methods (Wakelin et al. 2009).

Band intensity data from the DGGE gels were square root transformed and the similarity between samples determined by using the Kulczynski coefficient (Kulczynski 1928). This has been found to be robust in reconstructing nonlinear ecological responses (Faith et al. 1987) and is one of a number of closely related similarity coefficients (the ‘Bray-Curtis’ family) which have general suitability for ecological

studies (Clarke and Warwick 2001; Clarke et al. 2006). Ordination by principal coordinates analysis (PCO; Gower 1966) was used to display similarities in microbial community structure. The effects of Cu on the structure of the microbial communities in the presence and absence of medic substrate were tested using permutation-based multivariate analysis of variance (PERMANOVA+ for PRIMER; Anderson et al. 2008) using approaches described in Anderson (2001). In the design, addition of medic was used as a fixed treatment, and Cu levels were used as a fixed covariate and tested in interaction with medic (in a multivariate equivalent of analysis of covariance). For each test, 999 permutations were used to test for the effect, with the permuted residuals calculated under a reduced model (Anderson et al. 2008). The outcome is unaffected by the choice of type of sums of squares calculated because the design is balanced—both medic and basal treatments are conducted for the same set of Cu levels. This allows the three components of the ANOVA-type table (main effects of medic treatment and Cu levels and their interaction) to be tested independently.

The ecological distance (100-Kulczynski similarity) between the microbial community structures in each soil with respect to the control (Cu rate 0) was determined for soils with and without medic addition. This measure was used to determine the ecological shift (departure from the reference microbial community structure) of increasing Cu in soils with and without medic substrate addition. Regression lines were fitted through the data sets and the slope of the regression lines between the basal and medic-added data were compared and tested (statistical analysis conducted in GraphPad Prism version 5; GraphPad Software, San Diego). Comparison of intercepts, for this analysis of covariance, is not valid because of the reuse of the control (Cu=0) samples in calculating all ‘distances to control’ values, with separate controls for medic-added and basal conditions. Variation in control values could strongly affect the location of the two regression lines in relation to each other, but should not bias the comparison of regression slopes. Tests for equality of the latter are, however, interpreted suitably cautiously, the main purpose of the comparative regressions being to display the size and direction of any interaction effect between the Cu dose–response relationship and the addition of medic, where such interaction effects are demonstrated to exist by the (fully valid) PERMANOVA tests.

Results

Soil respiration over a Cu gradient

Basal soil respiration (no medic added) was low but monotonically increased with time (Fig. 1a). Across the data set, no trends were evident to suggest that increasing

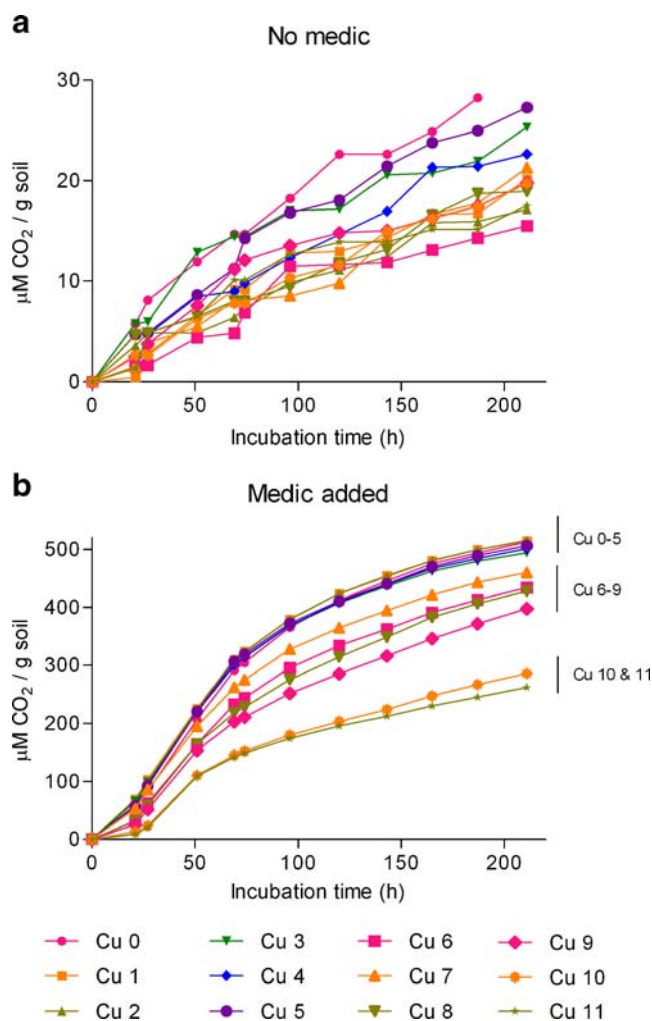


Fig. 1 a, b Soil respiration (CO_2 evolution) over time in soils containing a range of Cu levels. a The soils have had no added plant substrate (medic). b Medic stubble was added to the soil at 3% dry weight

Cu caused decreasing basal respiration (Fig. 1a). The relationship between CO_2 and Cu was further tested using the RELATE permutation test (for seriation), which showed no significant trend in the basal respiration curves with increasing Cu values ($\rho=0.043$; $P=0.349$).

In the medic-added incubations, soil respiration within a 21 h period generally exceeded the cumulative totals produced over 211 h for the basal treatments. Over the course of the experiment (i.e., 211 h), approximately 45% of the C from the medic was respired in the control soils. There was a clear trend whereby the total amount of CO_2 evolved steadily declined with increasing soil Cu levels (Fig. 1b). Notably, respiration in Cu 10 and Cu 11 ($>3000 \text{ mg Cu kg}^{-1} \text{ soil}$) fell discretely below the other Cu levels. In the medic-added treatments, the relationship between increasing Cu and decreasing respiration was found to be highly significant (RELATE test; $\rho=0.695$; $P=0.001$). Furthermore, soil respiration curves over the soil

Cu gradient followed classic dose–response models (Suppl. Fig. 1A) for all sampling times.

Bacterial community response

The community structure of soil bacteria significantly varied with respect to soil Cu (PERMANOVA; $P < 0.001$; Table 1); this is clearly seen in the distribution pattern of samples across the major (X) axis of the PCO ordination plot being driven by Cu (Fig. 2a). The addition of medic to the samples also caused a significant shift in the bacterial community structure ($P < 0.001$), which was of comparable effect size as the Cu gradient (component of variation data, Table 1).

The shift in community structure (ecological distance from the reference soil, Cu 0) showed strong and linear relationships with the LOG soil Cu levels (Table 1; Fig. 2b). The regression slopes were not strongly different and the evidence for a statistically significant difference was borderline (Fig. 2b and Table 1); i.e., only at about the 5% significance level for a test which, as explained earlier, is only approximate. The lack of significant interaction between the medic and Cu treatments in the PERMANOVA analysis (Table 1) suggests that there should not be a meaningful difference in slope here.

The community structure for the sample ‘medic added, Cu rate 8’ was highly anomalous and was omitted both from the PERMANOVA analysis, PCO plot and the regression analysis (Fig. 2a, b). However, the inclusion or removal of the data point did not alter the outcomes of the multivariate analysis (data not shown).

Archaeal community response

The archaeal community structure significantly varied with respect to soil Cu levels (PERMANOVA, $P < 0.001$; Table 1). The effects are largely due to changes in community structure occurring at Cu levels 10 and 11 (Fig. 3a). For both basal and medic-added soil, there are groupings of community similarity from Cu levels 0–9 (negative end of the X -axis) and then a large shift to the community types in soils Cu 10 and 11. It can be seen that the same patterns are present in both the basal and medic-added treatments; just the spread across the X -axis differs. The distinction between basal and medic-added conditions is also clearly established from the PERMANOVA analysis ($P < 0.001$, Table 1) and is apparent as a shift to larger values on the first PCO axis for the medic-added samples (Fig. 3a). However, in contrast to the bacterial plot of Fig. 2a, the total variance explained by the first two axes is not high in this case (57%), so the plot should be interpreted with some caution, more emphasis being placed on the PERMANOVA results based on the community similarity matrix itself, rather than its approximation in a lower dimensional ordination space. As with the bacterial community, there is no evidence for interaction between the medic addition and the effect of different Cu levels (Table 1), i.e., differences between the communities are a simple additive combination of the presence or absence of medic and the level of Cu dosing.

When the effects of Cu on shifting the archaeal community structure from the control state were examined (Fig. 3b), no significant linear responses were demonstrated (Table 1).

Table 1 Summary of PERMANOVA testing of copper and medic on microbial community structure and shift in ecological distance

Community structure ^a	Bacteria			Archaea			Fungi		
	CV ($\sqrt{}$)	F	<i>P</i>	CV ($\sqrt{}$)	F	<i>P</i>	CV ($\sqrt{}$)	F	<i>P</i>
Medic	20.53	20.53	0.001	15.85	7.11	0.001	13.04	5.94	0.001
Copper	17.94	10.54	0.001	12.59	8.71	0.001	22.89	8.59	0.001
Medic × Copper	4.91	1.71	0.190	-5.22	0.34	0.872	15.50	4.49	0.002
Residual	19.68			22.22			28.13		
Ecological distance from reference community ^b									
		R^2	<i>P</i>		R^2	<i>P</i>		R^2	<i>P</i>
Positive medic regression		0.775	<0.001		0.07	0.706		0.581	0.006
Negative medic regression		0.907	<0.001		0.258	0.111		0.863	<0.001
		F	<i>P</i>		F	<i>P</i>		F	<i>P</i>
Compare slopes		4.43	0.05		0.693	0.416		8.276	0.010

^a Community composition (PCR-DGGE banding profiles) of the bacterial, archaeal and fungal communities. Band intensity data (=species abundance) was square root transformed and resemblance between samples determined using Kulczynski’s similarity coefficient. Community structures were compared using permutation-based multivariate analysis of variation (PERMANOVA) with medic as a fixed factor and Cu as a covariate. CV($\sqrt{}$) is the square root of the component of variation (Anderson et al. 2008), a measure of the effect size in units of the community dissimilarities (negative values indicate effectively zero components).

^b Departure in community similarity from the reference community type (Cu rate 0). This was calculated as 100-Kulczynski similarity (=dissimilarity).

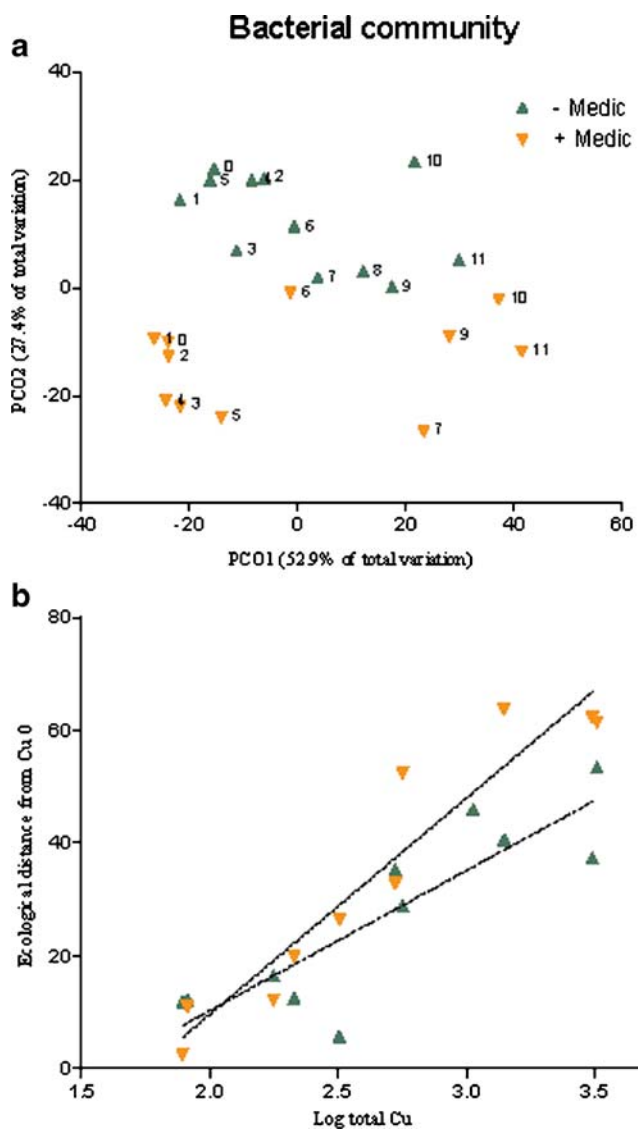


Fig. 2 **a, b** Response of the soil bacterial community to medic addition over a range of soil Cu values. **a** Principal coordinates analysis (PCO) ordination plot showing similarity in community structure among all samples. **b** Shift in bacterial community structure from the reference (Cu 0) community type over the Cu range in soil with and without medic addition. Bacterial community structures were determined by PCR-DGGE analysis of the 16S rRNA gene. Abundance data (band intensity) were square root-transformed prior to analysis. Similarity in community structure was based on the Kulczynski coefficient. Ecological distance = 100 - Kulczynski similarity. An outlying data value (Cu 8, medic added) was excluded from the analysis

Fungal community response

The soil fungal community was significantly affected by both Cu and medic addition (clearly seen in Fig. 4a), including a significant interaction (PERMANOVA interaction term, $P=0.002$; Table 1). The interaction effect is very evident from the PCO ordination (Fig. 4a), though again some caution is required in interpreting the PCO plot since the first two axes account for only slightly more than half of

the total variation. What appears clear is that for the upper half of the soil Cu values ($\geq \text{Cu } 6$), the addition of medic resulted in the occurrence of highly similar fungal community types, whereas the same close community similarity at high Cu rates was not evident in the fungal community in the basal system. This was formally tested using the PERMDISP test (Anderson 2006) for equality of multivariate dispersion for the two groups (i.e., samples from Cu levels 6 to 11 for the medic and basal treatments; deviations tested from the centroid). The results showed that the dispersion of fungal medic and basal communities over this Cu range were significantly different ($P=0.026$), with the average deviation in the basal treatment of 29.0 (SE=1.6) and only 15.2 (SE=3.7) in the medic-added treatment.

This is also a feature of the relationship between Cu values and the shift in fungal community structure from the

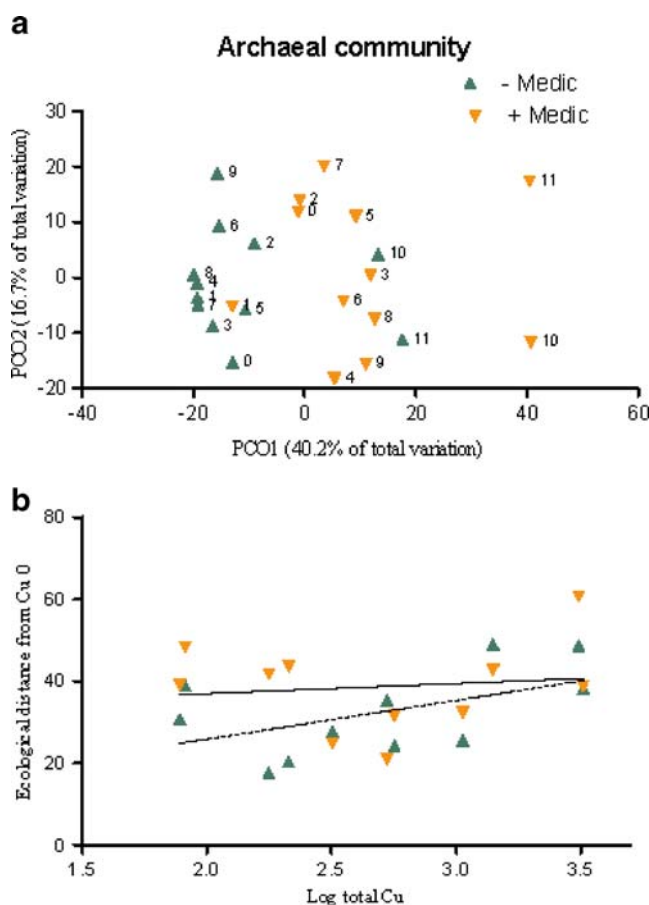


Fig. 3 **a, b** Response of the soil archaeal community to the effects of medic addition over a range of soil Cu values. **a** Principal coordinates analysis (PCO) ordination plot showing similarity in community structure among all samples. **b** Shift in archaeal community structure from the reference (Cu 0) community type over the Cu range in soil with and without medic addition. Archaeal community structures were determined by PCR-DGGE analysis of the 16S rRNA gene. Abundance data (band intensity) were square root-transformed prior to analysis. Similarity in community structure was based on the Kaczynski algorithm. Ecological distance = 100 - Kulczynski value

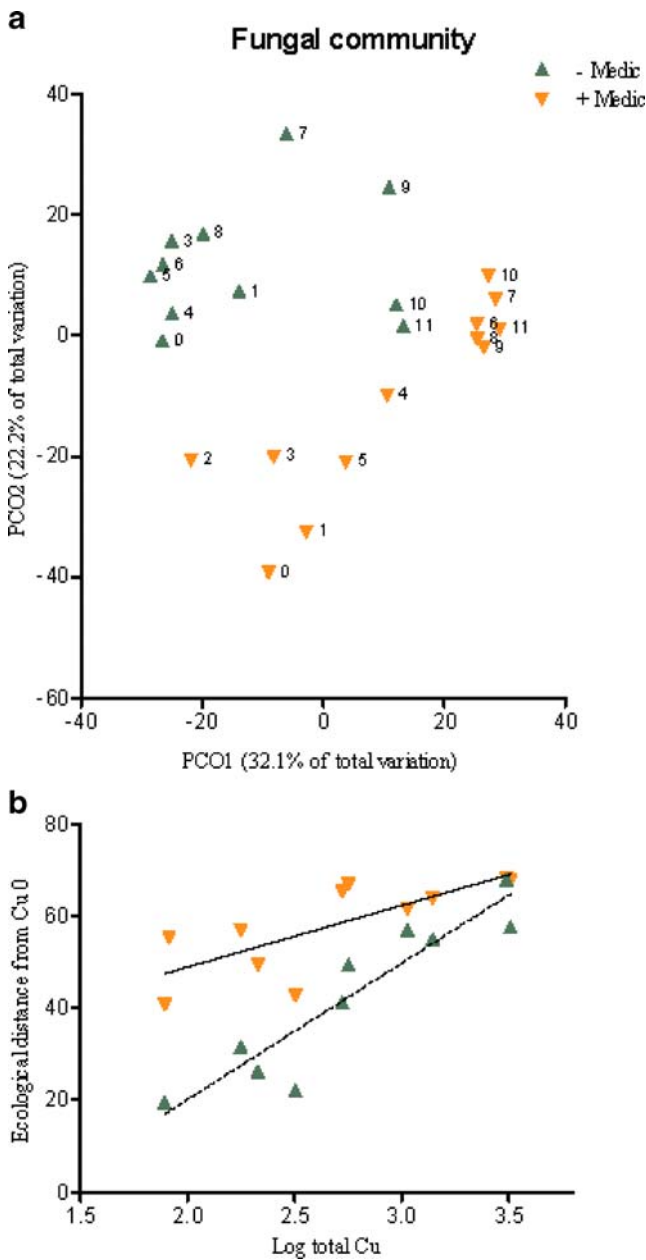


Fig. 4 a, b Response of the soil fungal community to the effects of medic addition over a range of soil Cu values. **a** Principal coordinates analysis (PCO) ordination plot showing similarity in community structure among all samples. **b** Shift in fungal community structure from the reference (Cu 0) community type over the Cu range in soil with and without medic addition. Fungal community structures were determined by PCR-DGGE analysis of the ITS gene. Abundance data (band intensity) were square root-transformed prior to analysis. Similarity in community structure was based on the Kulczynski algorithm. Ecological distance=100-Kulczynski value. An outlying data value (Cu 2, no medic) was excluded from the analysis

control conditions. The regression lines have highly significant slopes for both basal and medic-added soils (Fig. 4b; Table 1), and those slopes are significantly different from each other ($P < 0.01$, Table 1). Thus, at low Cu levels, the addition of medic changed the fungal

community more strongly from control conditions (Cu 0) than was the case for the basal community. At the highest Cu values, the communities appear to diverge equally strongly from control conditions under both presence and absence of medic (Fig. 4b).

The community structure of the sample ‘no medic, Cu rate 2’ was highly anomalous. It has been omitted both from the PCO plot and the regression analysis (Fig. 4a and b).

Discussion

Early decomposer processes (medic mineralisation) are impacted by soil Cu

The early phase (0–9 days) decomposition of *Medicago* detritus was significantly influenced by the background level of Cu in soil and followed classic dose–response relationships over all the time points investigated. The results are important for several reasons. Firstly, the toxicity is based on increasing doses of Cu added to the soil, as a single application, ~6 years ago. Thus, the effects of this Cu addition are both enduring over time and also retain toxicological behaviour over the entire Cu application range. Following from this, the extent of microbial adaptation to the presence of soil Cu, from a functional perspective, appears to be limited. If significant adaptation of the microbial community had occurred by mechanisms such as species selection and replacement, it would be expected that restoration of decomposer activity would occur, particularly given that plant material is the primary resource driving microbial community dynamics. Lastly, microbially mediated mineralisation of newly applied organic matter is of the most fundamental of soil ecosystem functions. Organic matter (carbon) cycling is directly coupled with numerous other nutrient cycles, drives soil stability through aggregate formation and so forth (Swift et al. 1979).

Although substrate-induced respiration was affected by soil Cu levels, basal respiration was not. Similar results have been found previously (Broos et al. 2005), demonstrating lack of sensitivity of microbial basal respiration to increasing soil Cu. This raises a number of questions with respect to both ecotoxicological interpretation of soil Cu EC₅₀ data and also how Cu affects soil biota and its interaction with organic matter. In particular, the toxicological impacts of Cu appear to be strongest on the fast-growing (*r*-strategist) microbiota capable of rapidly responding and capturing fresh resources. We propose, therefore, that substrate-induced EC₅₀ values capture only a discrete sub-component of the microbial community involved in rapid carbon turnover. Microbial interactions with more recalcitrant forms of soil C, with which basal respiration is assumed to be occurring, was

unaffected by soil Cu levels even after 6 years exposure. Accordingly, it can be hypothesised that there is an interaction between C resource quality and the toxicological impact of Cu on microbial resource use. It is not surprising, therefore, that the most sensitive tests for soil metal toxicity to microbial processes are those reliant on rapid mineralisation of simple sugars such as glucose (Broos et al. 2005). What are the implications, therefore, for long-term cycling of soil organic matter? To date, we cannot find any comprehensive analysis of soil C fractions in field soils containing a Cu dose response. However, soil Cu levels are related to carbon held in the microbial biomass (Brookes and McGrath 1984), correlate with soil organic C (Valsecchi et al. 1995; Dai et al. 2004) and total C levels (Coughtrey et al. 1979). In addition, numerous studies have shown the impacts of Cu and other heavy metals on the fate of soil C, particularly into the humified fraction (Russell and Alberti 1998; Gillet and Ponge 2002; Filzek et al. 2004). It has even been proposed that soil organic matter profiles could be used as ecotoxicological tool to estimate ecosystem damage by heavy metals (Beyer 2001). Regardless of the effects (or otherwise) of Cu on soil C partitioning into various soil pools (labile, refractile, humic, etc.), the kinetics underpinning the rate of movement and transformation between pools is clearly altered.

Long-term Cu exposure changes soil microbial community structure

Structural change in the bacterial, archaeal and fungal communities were evident across the soil Cu gradient. These changes were linear when the shift in community structure (ecological distance=100-Kulczynski value) from the control (Cu rate 0) community assemblage was plotted against the LOG of the total soil Cu. Furthermore, testing by PERMANOVA established the significance of Cu on affecting microbial community structure. As such, the results here are important, as they show that the fundamental biodiversity within soil ecosystems, across all three domains, was perturbed from the expected 'optimal' community structure (i.e., community structure at Cu rate 0). If we assume that the community within the control soil represent the taxa most fit to the soil niche, then it follows that the high selective pressure exhibited by Cu overrides the wider niche-selective properties (the underlying soil physicochemical properties). The results of the medic decomposition study show that the functioning of the Cu-adapted community is compromised. This may be because the Cu-adapted community is less adept at functioning within the soil compared to the optimal community—its presence is not based on the most suitable taxa for that soil, but the taxa most tolerant to Cu. This drift towards similar microbial community profiles with respect to metal addition has been seen before (Bamborough and Cummings 2009).

Due to a high level of functional redundancy across species, direct relationships between diversity of microorganisms and processes such decomposition of organic matter are rare (Nannipieri et al. 2003; Moore et al. 2004; Hättenschwiler et al. 2005). As such, a reduction of biodiversity within this functional group is unlikely to be directly linked to decomposition (Nannipieri et al. 2003; Moore et al. 2004; Hättenschwiler et al. 2005). Rather, the presence of Cu itself may exert a strong physiological burden on the community which affects decomposition. This has been measured previously, whereby the addition of Cu to soil increases the metabolic quotient (qCO_2) or energy used per unit of CO_2 respired during decomposition (Giller et al. 1998; Merrington et al. 2002). These hypotheses may be experimentally tested by establishing Cu-adapted community types in low Cu soils (via sterilisation and soil transfer) and characterising the decomposer-capacity of the community in a low Cu environment.

Microbial community responses to plant residue addition over a Cu exposure gradient

Plant matter (detritus) plays a central role in community organisation and trophic structure in soil ecosystems (Moore et al. 2004). The rapid response to new energy resources entering soil is a fundamental property of soil ecosystems. As such, the effects of strong stress, such as Cu, within soil may affect the responsive nature of consumer communities. Our data showed that addition of medic residue to soil resulted in significant shifts in bacterial, archaeal and fungal communities ($P < 0.001$). Furthermore, the effects of increasing soil Cu on community structure were also clearly evident within soils in which medic was added or not. However, for the bacterial and archaeal communities, these effects were not interactive—with increasing soil Cu, there was increasing shift in soil community structure in soil with and without medic.

The response of the soil fungal community, however, was quite different to that measured for the bacteria and archaea. With addition of medic, a large shift in community structure occurred at low Cu levels, but this shift in community type was increasingly diminished with increasing soil Cu. At high Cu levels, there was reasonable convergence of fungal community structure, and community dispersion was significantly smaller than at low soil Cu levels. This interaction effect between Cu and medic ($P = 0.002$) is important, as it shows that the increasing soil Cu affects the capacity of the fungal community to respond to addition of new resources. This is important as fungi are primary agents of plant material decomposition and pioneer early transformation of cellulose, hemicellulose, lignocellulose, lignin, etc. The reduction in microbial respiration (substrate induced) under increasing soil Cu is consistent with the observed impacts

on the fungal community, and this is in accordance with the role of fungi in primary decomposition of plant matter.

Outlook on wider effects on ecosystem function

With increasing soil Cu, microbial communities show a large degree of compositional alteration. The shift in species is associated with a decrease in decomposer activity; however, we cannot say whether this is due to a higher stress within the community itself (metabolic quotient is shifted/reduced fitness) or reduced inherent capability of the replacement species to decompose plant material. Over time, the shifts in microbial community structure and decomposer function are likely to lead to strong decoupling of associated nutrient cycles, trophic level interactions and food web stability (Srivastava et al. 2009). Ultimately, these will affect energy transfer and nutrient flow through to plants and consequently affect interactions with herbivores, pathogens, symbionts and change in the ecosystem as a whole (van der Putten et al. 2009).

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