Impact of Long Term Sewage Sludge Additions to Biological Function in Scottish Soils

Background

Results from the UK Long Term Sewage sludge Experiment (LTSE; *Long-term Sludge Experiments - Phase III report.2007. Defra*) indicated that certain soil microbial populations were reduced below detectable limits after sludge application. It was reported that nitrogen fixing rhizobium bacteria were susceptible to toxicity from components of sludge and this was a long term effect.

The results have important implications for Scotland's most versatile agricultural soils which cover a small percentage of the total land surface but receive the majority of the permitted sewage sludge applications to land. Recovery of sewage sludge to agricultural land is a main component of Scottish Water's draft sludge strategy. The results from the long-term study may have important implications both for this strategy and for the land on which sludge is used. The Scottish Government needs to determine if the reported impacts affect the ability of soils to perform basic biological functions such as decomposition and nutrient cycling in the long term.

This project focussed on the impacts of sludge additions on biological ***function*** as opposed to those which affect biological ***diversity***. Where appropriate observations on the links and synergies between functional groups and diversity are elaborated.

Objectives

Obj 1. To conduct a series of laboratory experiments on soils from the two Scottish sites at Auchincruive and Hartwood used in the UK long term sewage sludge trials to evaluate biological function relevant for soil fertility.

We met this objective by sampling from plots at the Hartwood and Auchincruive sites. As adverse effects have only been recorded in relation to Zn- and Cu- rich sludges only these plots plus controls and long term build-up plots (low and metal rich) were sampled (Treatments 1-11, 16-19). This computed as 15 treatments x 3 reps =45 plots per site and 90 plot samples in all.

These samples were used for laboratory measurements as detailed below and a subset from Hartwood was used for setting up microcosms and pot experiments with clover plants.

Obj 2. To infer from the laboratory data whether sludge applications to land affect only taxonomic diversity or whether they impact on the ability of these soils to perform biologically mediated functions which support crop growth.

A variety of functional tests were performed to determine if C and N cycling has been affected and the results compared to existing and new information on the taxonomic effects.

Methods

Within the constraints of the contract duration (9 months) and the window for field soil sampling, experiments were designed to evaluate the ability of soil organisms to:

- decompose organic matter
- re-cycle nutrients, particularly nitrogen

Biological functions relevant for agricultural productivity include the ability to supply nutrients (N and P, and trace elements) via symbiotic associations, good soil structure, the control of pests and diseases. The results were evaluated for any long term detrimental effects on soil biological function in relation primarily to soil nutrient supply and cycling. Analysis of the major groups of organisms performing decomposition functions and N fixation were noted and discussed in the context of *inherent redundancy* in the soil microbial biomass.

While the specification mentions the methods should include the following (Text Box 1 Column 1) we recommended varying this to take account of new approaches (Text Box 1 Column 2) and the fact that some of these measures have been or are already being measured at the sites. These recommendations were accepted at the inception meeting.

Text Box 1 - Specified and proposed (and accepted) methods.

Specified methods:	Accepted Methods:
• *Biolog (or an equivalent method) to evaluate a wide range of plant derived materials which can be decomposed by the microbial populations in these soils and to note any statistically significant anomalies.*	• Catabolic diversity using MicroResp (Campbell *et al*., 2003) which gives better discrimination than both Biolog (Campbell *et al*., 2003) and the Degens and Harris, (1997) multiple substrate induced respiration methods (see Lalor *et al*., 2007).
• *Some measure of nitrogen turnover such as mineralisation, nitrification and/or N fixation using established methods.*	• The SGRERAD funded WP3.2 already measures N mineralisation and root elongation and therefore we measured

	nitrification and N fixation using established methods.
• *An evaluation of the major groups of organisms performing these functions and any implications for soil fertility.*	• The major groups of organisms were evaluated by an analysis of the phospholipids fatty acid profiles of the soil.

Decomposition

Decomposition processes are fundamentally important for nutrient cycling. The incorporation of plant derived material into the soil and subsequent turnover of organic matter, the transformation of organic pollutants, soil buffering capacity and the maintenance of soil structure are all affected by decomposition rates.

To test the soil's ability to decompose litter, root exudates and complex plant components and exudates as well as common soil pollutants such as biocides and organic pollutants a range of ^{14}C-labelled substrates, including ryegrass litter and xenobiotics, were used in the MicroResp™ apparatus (Campbell *et al*., 2003). The use of ^{14}C labelled substrates to determine catabolism without the masking signal of basal respiration and the use of a wide range of substrates allowed us to measure both broad- and narrow-niche functions in the soil population. It has been shown that separating broad- and narrow-niche functions in this way to look at soil nutrient dynamics is useful in determining how soil communities are affected by perturbation and the impact on microbial diversity (Girvan *et al*., 2005).

N cycling

Rhizobium numbers have been shown to be reduced in Hartwood soil since 1998 (Chaudri *et al*., in press) and more recently at Auchincruive. In both cases effects were seen only in the Zn-rich plots (unpublished 2007 data). Consequently, the Zn-rich plots at the two sites were used for additional functional measures relative to N fixation. As the plots are maintained free of clover it is inappropriate to measure N fixation in the field or laboratory without seeding with clover plants. Seeding the field plots with clover would compromise the long term integrity of the experiments. Consequently laboratory and pot experiments were used to assess whether soil functional capacity to nodulate has been impaired. This was done by growing clover plants in soils most affected by Zn-rich sludge and assaying them for the recovery and N fixation potential (acetylene reduction assay) of Rhizobium species. This included an additional laboratory treatment using control soil to re-inoculate the highest Zn-rich soils from Hartwood with Rhizobium to determine if re-inoculation can remediate loss of diversity and restore function.

N mineralisation was already measured on Hartwood and Auchincruive as part of the LTSE protocols and these data are presented here for comparison. However we also measured potential nitrification by ammonium sulphate addition using the ISO standard method.

Major Groups of organisms

The major groups of organisms were evaluated by an analysis of the phospholipid fatty acid profiles of the soil. The cycling of N, P and trace elements (micronutrient) are important functions in such soils receiving waste water sludge. The mediation of P supply in grasslands is primarily undertaken via symbiotic association with arbuscular mycorrhizal fungi (AM fungi) which have hitherto not been measured at any of the LTSE sites. Consequently we measured both the phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) to determine broad taxonomic groups of fungi, bacteria and actinobacteria (in the PLFA fraction) and AM fungal biomass (in the NLFA fraction; Olsson *et al*., 1995). The extraction, fractionation and analysis of lipids is a sensitive method for determining metal effects (Bååth *et al*., 1998).

Results

Major Groups of microorganisms - Lipid analysis

Phospho- and neutral lipids were extracted from all soils. There was no significant difference between treatments at either site for total PLFA, bacteria and fungal markers and for the NLFA C16:1?5 at Auchincruive (Table 1). At Hartwood, there was a significant difference in the fungal:bacterial (FB) ratio (P=0.05) between the Cu levels (50, 100, 150 & 200) and the control soil. NLFA C16:1?5c, indicative of AM fungi, was significantly different at Hartwood (P=0.05) between the metal treated soils (Zn (150, 250 350,450, LTB) & Cu (50, 100, 150, 200) and the control and undigested treatments.

Significant correlation was found between NLFA C16:1?5c and Cu in both soils and the fungal:bacterial ratio and Cu in Hartwood soil (Table 2; Fig. 1 & 2). With Zn, correlation with NLFA C16:1?5c and the fungal:bacterial ratio was only found in the Hartwood soil. Fungal PLFA markers but not bacterial PLFA significantly correlated with Cu but not Zn treated soils at Hartwood. No significant correlation was found Auchincruive for the fungal or bacterial PLFA markers.

Significant correlation was found between % N and bacteria PLFA signature in both soils (comparing all soil treatments) at both sites and with Fungal:bacterial ratio (Hartwood) and total PLFA (Auchincruive) (Table 3).

Using canonical multivariate analysis some clustering of treatments was found (Fig. 3). The more concentrated metal treatments (Zn 350 & 450 & Cu 150 & 200) were separated from the undigested and digested low metal concentrations in Hartwood (Fig. 3b). With Auchincruive soil (Fig. 3a) there was some separation between the Cu and Zn metal treatments regardless of metal concentration.

Table 1. Mean total, bacterial and fungal PLFA, the fungal:bacterial PLFA (FB) ratio, and C16:1?5c from NLFA for site × treatment with the significance of the ANOVA (F pr) and least significant difference at the 5% level (LSD). Different letters between values in the same column signify significant differences (P<0.05). Where there are no letters, there are no significant differences between the soil treatments. LTB = Long term build up.

Treatment	NLFA C16:1?5c		PLFA total		PLFA bacteria		PLFA fungi		FB ratio	
	Auchincruive	Hartwood	Auchincruive	Hartwood	Auchincruive	Hartwood	Auchincruive	Hartwood	Auchincruive	Hartwood
Control	5.46	8.604 b	100.1	189.3	47.6	90.4	14.0	28.4	0.31	0.32 c
Undigested	2.02	3.916 ab	135.1	201.1	65.7	103.9	17.1	26.6	0.26	0.276 abc
Undigested LTB	4.08	5.57 ab	125.8	205.0	62.1	97.2	17.3	30.0	0.28	0.308 bc
Cu LTB	5.41	6.218 ab	127.2	220.7	64.3	115.4	16.1	27.9	0.28	0.246 abc
Cu 50	5.54	3.258 a	163.2	242.9	85.9	133.7	17.0	28.0	0.21	0.207 ab
Cu 100	3.85	2.422 a	154.8	207.0	85.4	111.2	16.6	22.6	0.27	0.203 ab
Cu 150	3.39	1.792 a	137.0	213.5	66.2	116.5	17.9	22.5	0.28	0.194 a
Cu 200	2.46	2.469 a	138.9	203.3	70.9	114.3	17.1	21.5	0.26	0.191 a
Digested low metal	4.43	2.832 a	148.0	296.1	76.9	158.1	17.5	37.8	0.23	0.247 abc
Digested low metal LTB	4.88	3.828 ab	129.3	261.7	65.2	140.6	15.8	32.5	0.24	0.231 abc
Zn LTB	5.36	2.783 a	95.5	174.7	40.8	83.9	14.9	24.8	0.37	0.295 abc
Zn 150	5.72	2.317 a	100.8	257.6	49.9	142.4	13.0	30.0	0.28	0.211 abc
Zn 250	5.42	3.475 a	117.7	249.7	57.2	132.3	15.2	30.6	0.27	0.237 abc
Zn 350	3.24	2.088 a	139.2	228.6	72.7	124.2	16.0	26.7	0.23	0.215 abc
Zn 450	2.27	1.544 a	139.0	198.3	75.8	105.7	14.8	23.4	0.20	0.234 abc
F pr	0.073	<0.001	0.419	0.251	0.592	0.119	0.404	0.189	0.485	0.002
LSD	2.74	2.78	55.22	81.62	40.27	46.31	3.926	10.41	0.119	0.0621

Figure 1a **.** Correlation of total Cu and Zn with PLFA for Hartwood LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; i) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◇), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
metal LTB (△), Zn LTB (□), Zn 150 (■), Zn 200 (■), Zn 250 (■), Zn 450 (■). Each
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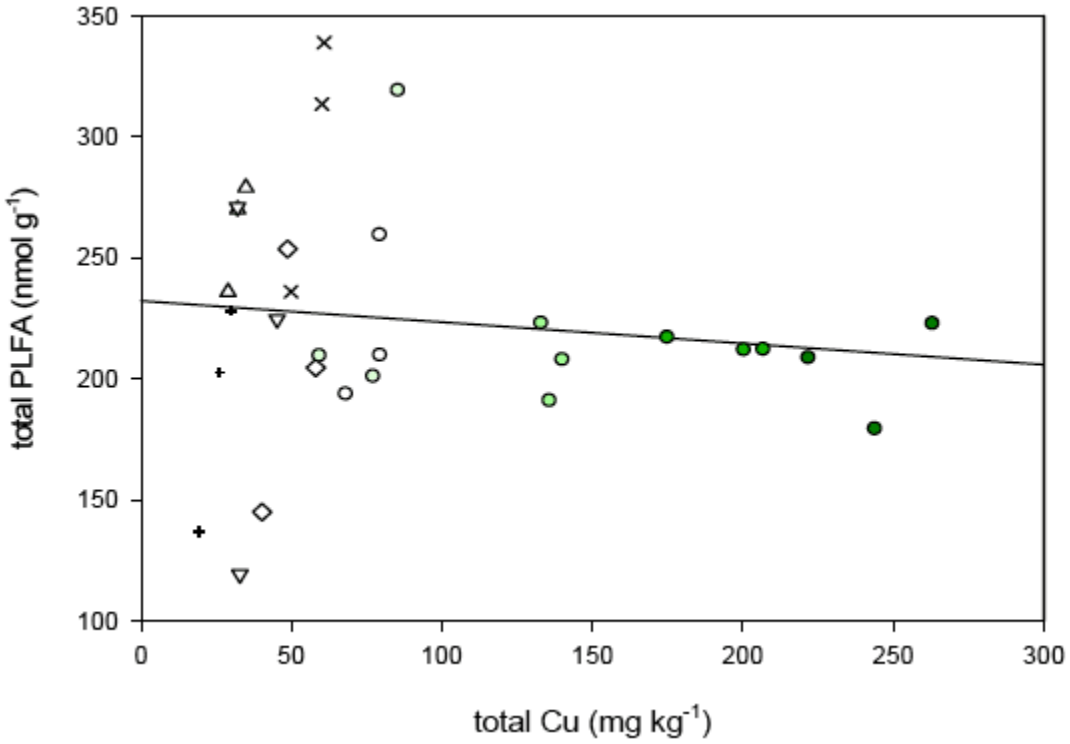

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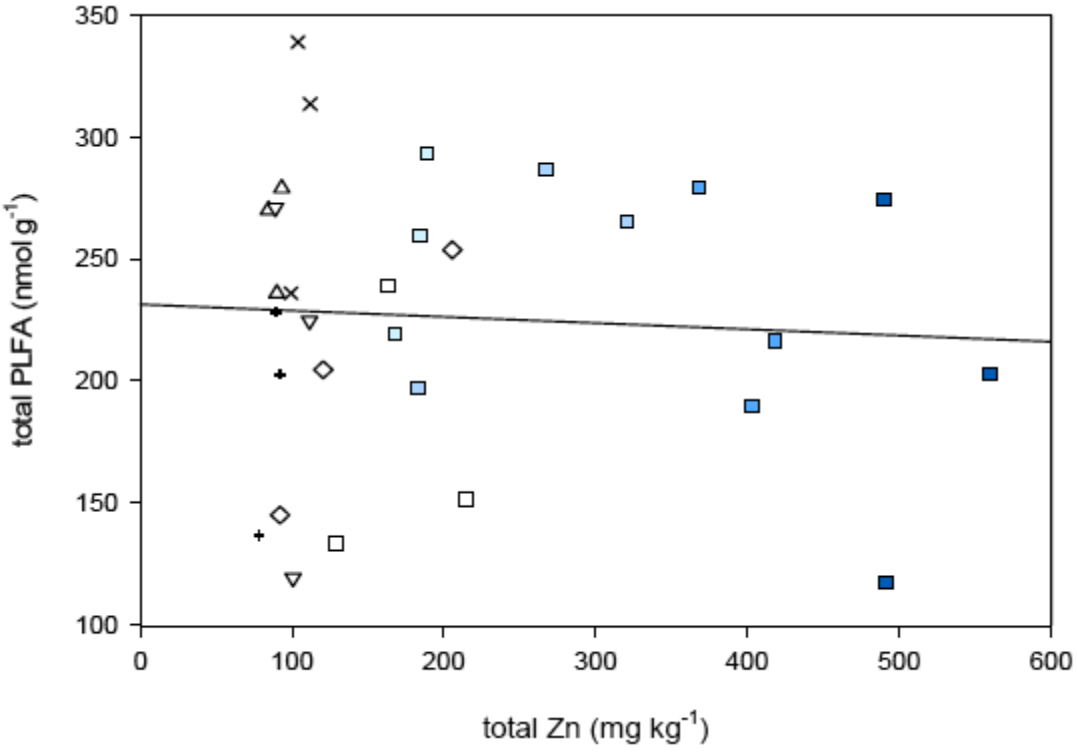

Figure 1b **.** Correlation of total Cu and Zn with fungal PLFA for Hartwood LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◈), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
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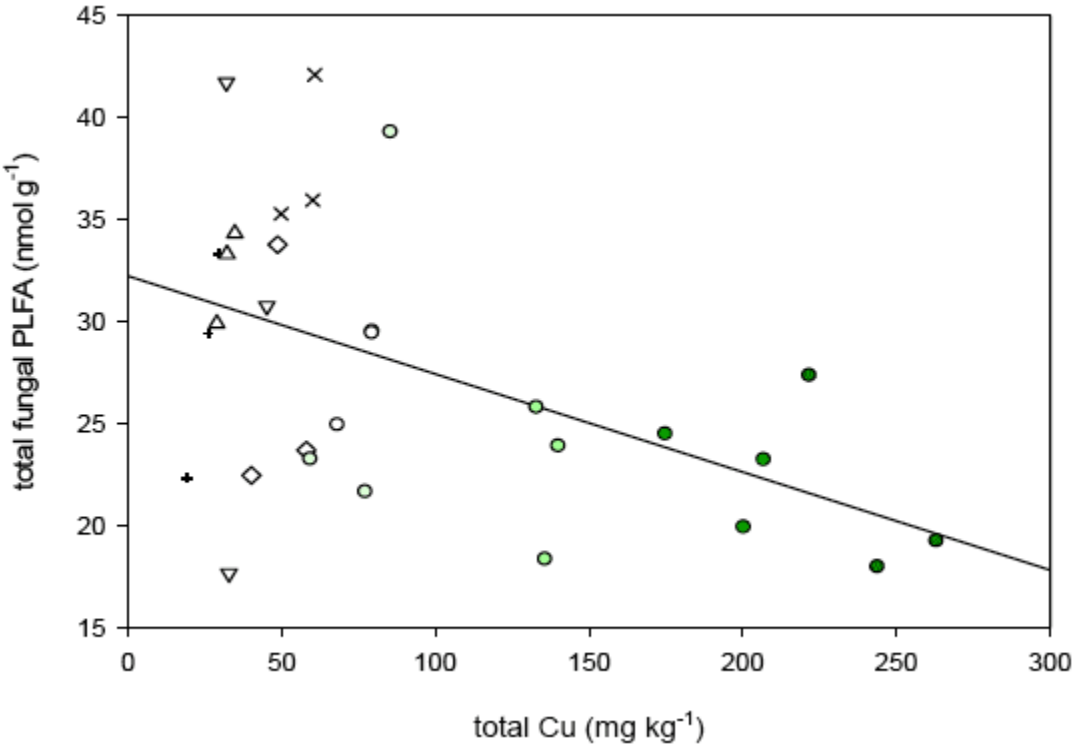
45
40
35
30
25
20
15
0
50
100
150
200
250
300
total fungal PLFA (nmol g-1)
total Cu (mg kg-1)

ii)

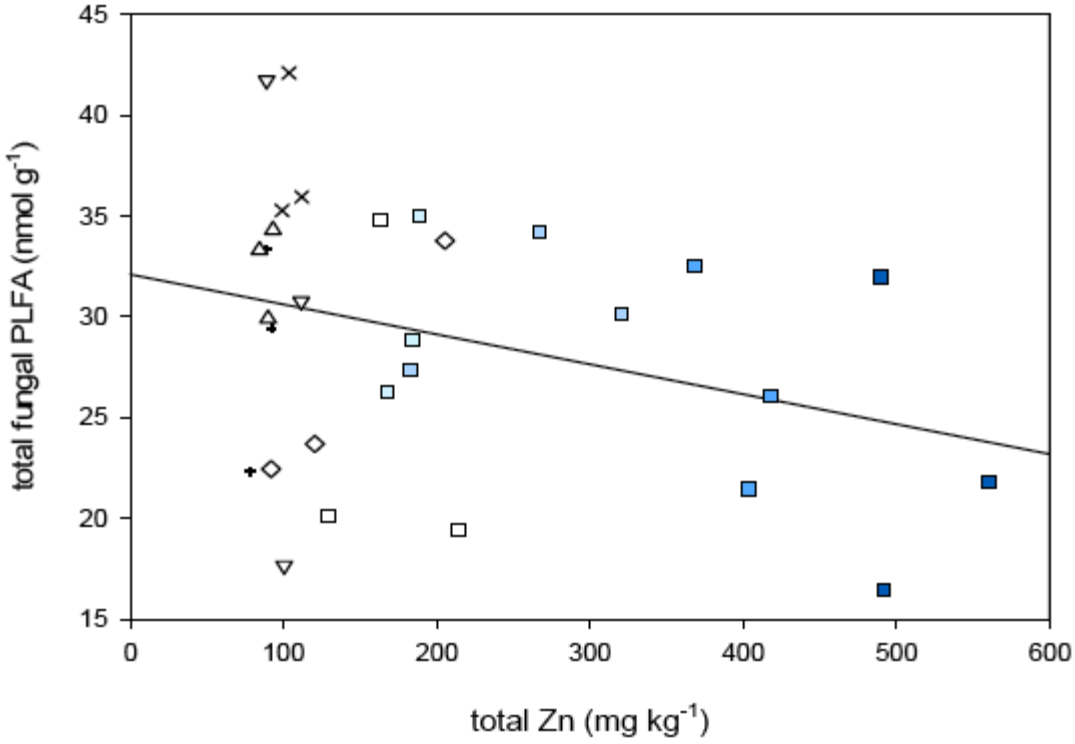
45
40
35
30
25
20
15
0
100
200
300
400
500
600
total fungal PLFA (nmol g-1)
total Zn (mg kg-1)

Figure 1c **.** Correlation of total Cu and Zn with NLFA C16:1?5c lipid (indicative of AM fungi) for Hartwood LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◈), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
metal LTB (△), Zn LTB (□), Zn 150 (■), Zn 200 (■), Zn 250 (■), Zn 450 (■). Each
replicate block is shown as a separate symbol.

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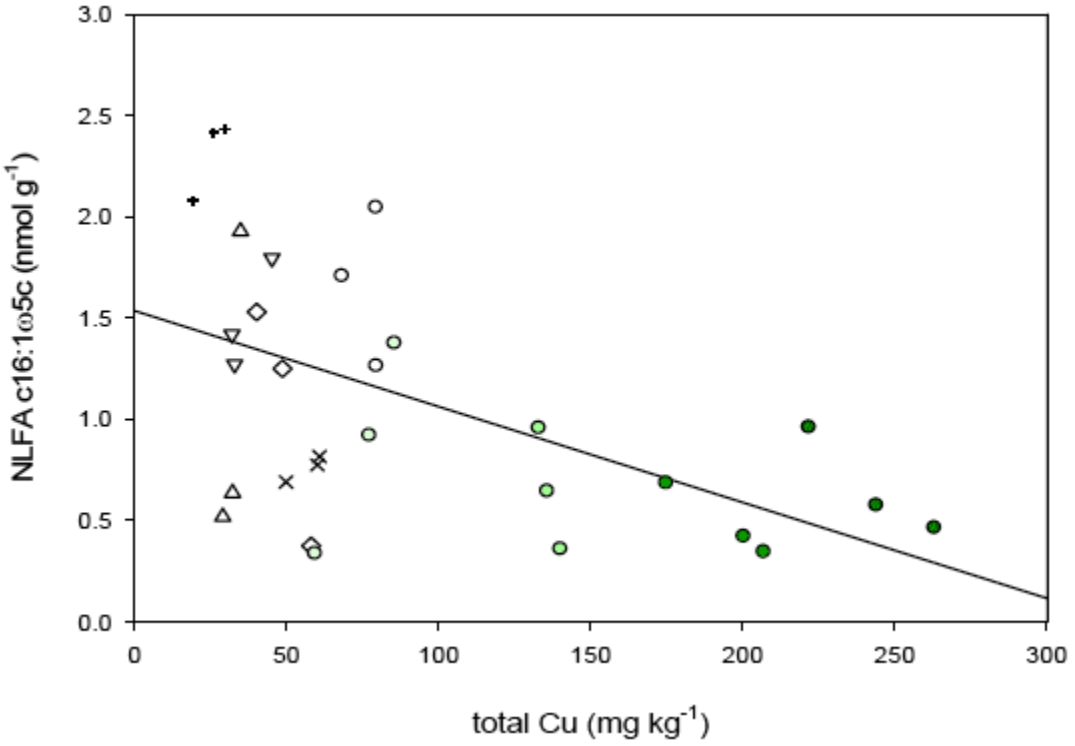

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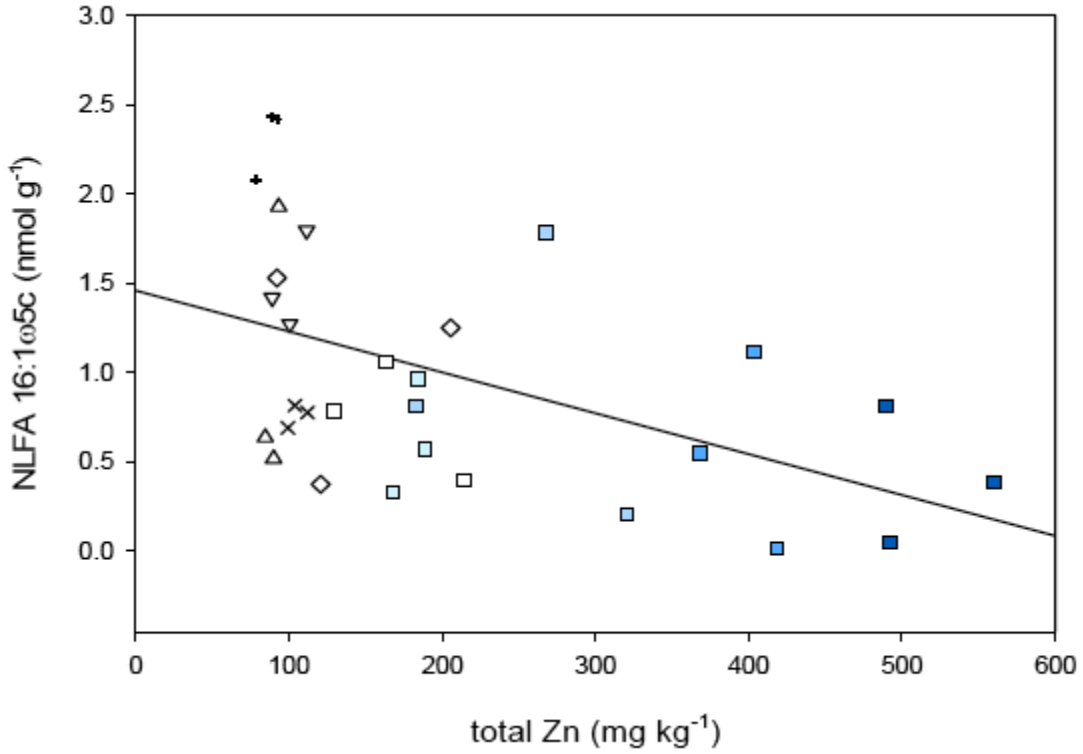

Figure 1d **.** Correlation of total Cu and Zn with bacterial PLFA for Hartwood LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◇), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
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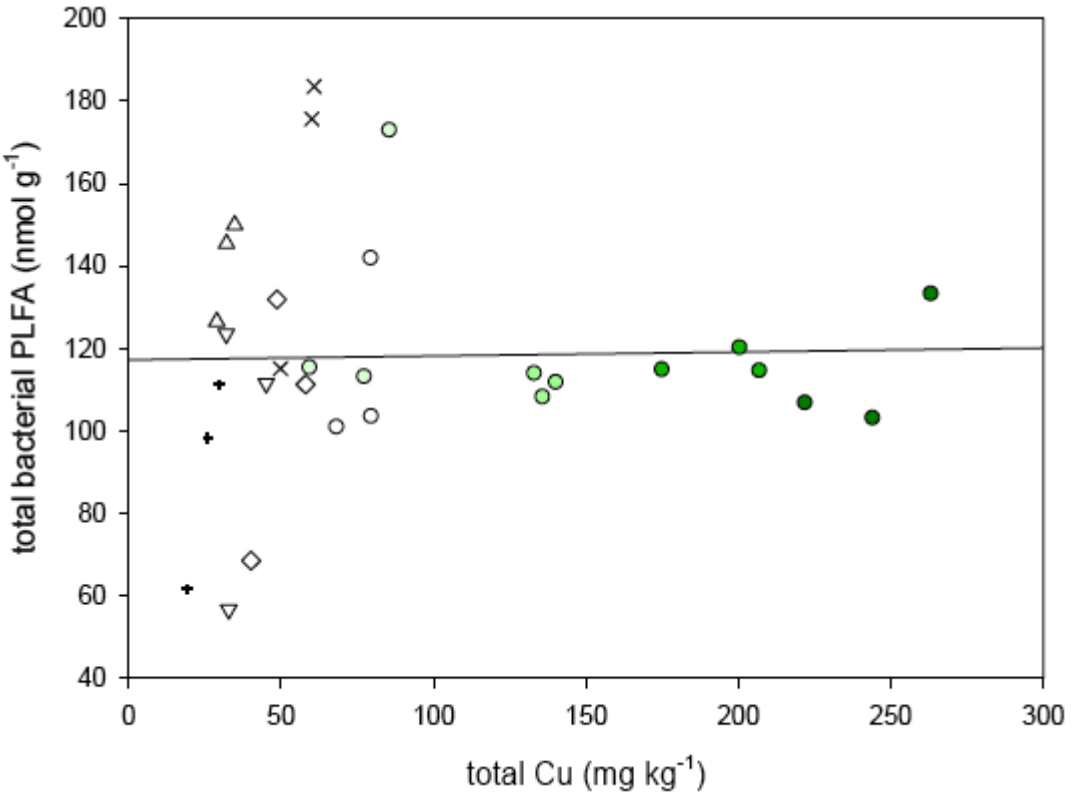
total bacterial PLFA (nmol g-1)
total Cu (mg kg-1)
200
180
160
140
120
100
80
60
40
0
50
100
150
200
250
300

ii)

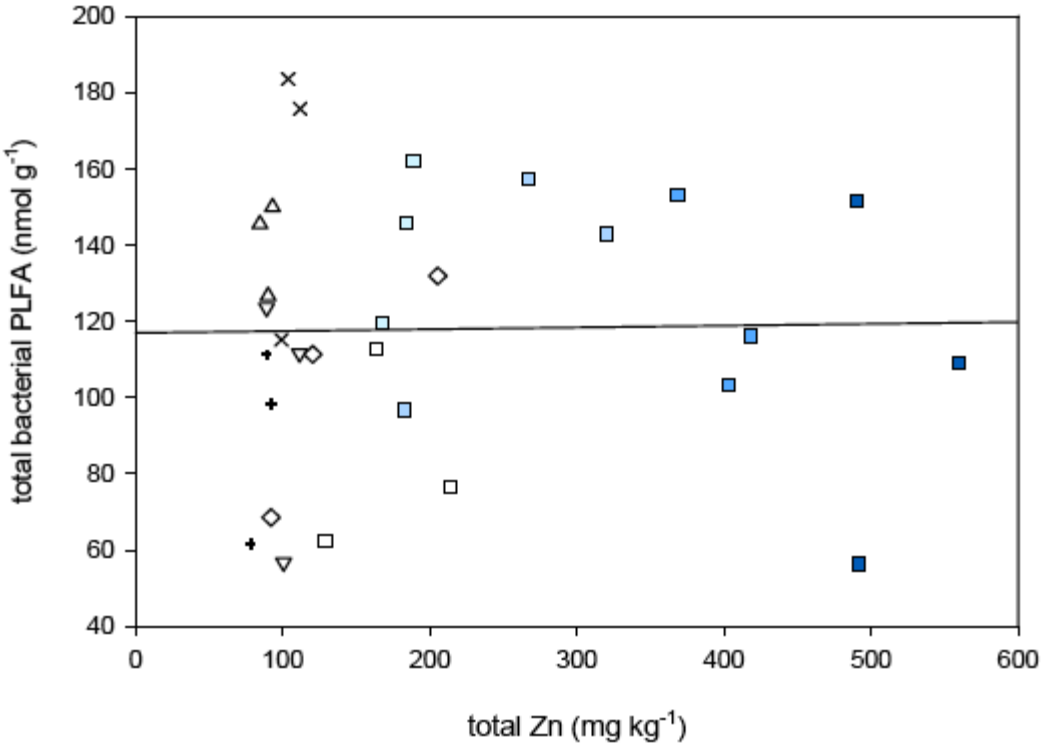
total bacterial PLFA (nmol g-1)
total Zn (mg kg-1)
200
180
160
140
120
100
80
60
40
0
100
200
300
400
500
600

Figure 1e . Correlation of total Cu and Zn with fungal:bacterial ratio for Hartwood LTSE on selected soil treatments . i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◈), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
metal LTB (△), Zn LTB (□), Zn 150 (■), Zn 200 (■), Zn 250 (■), Zn 450 (■). Each
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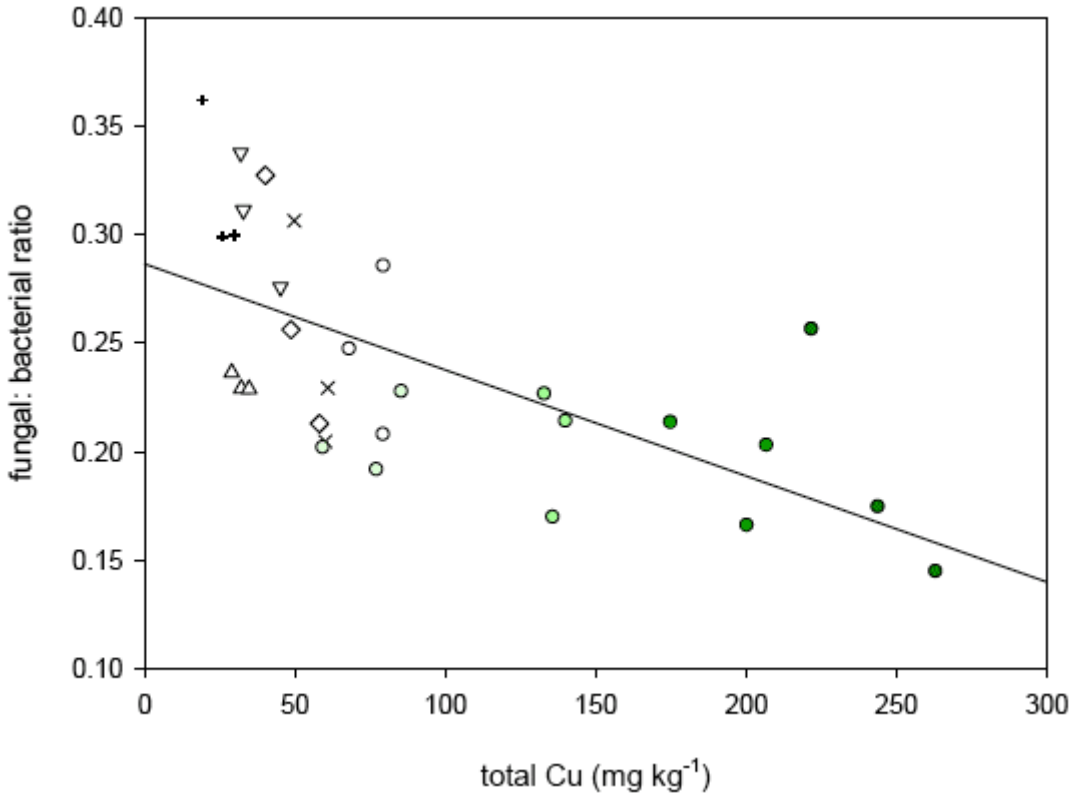

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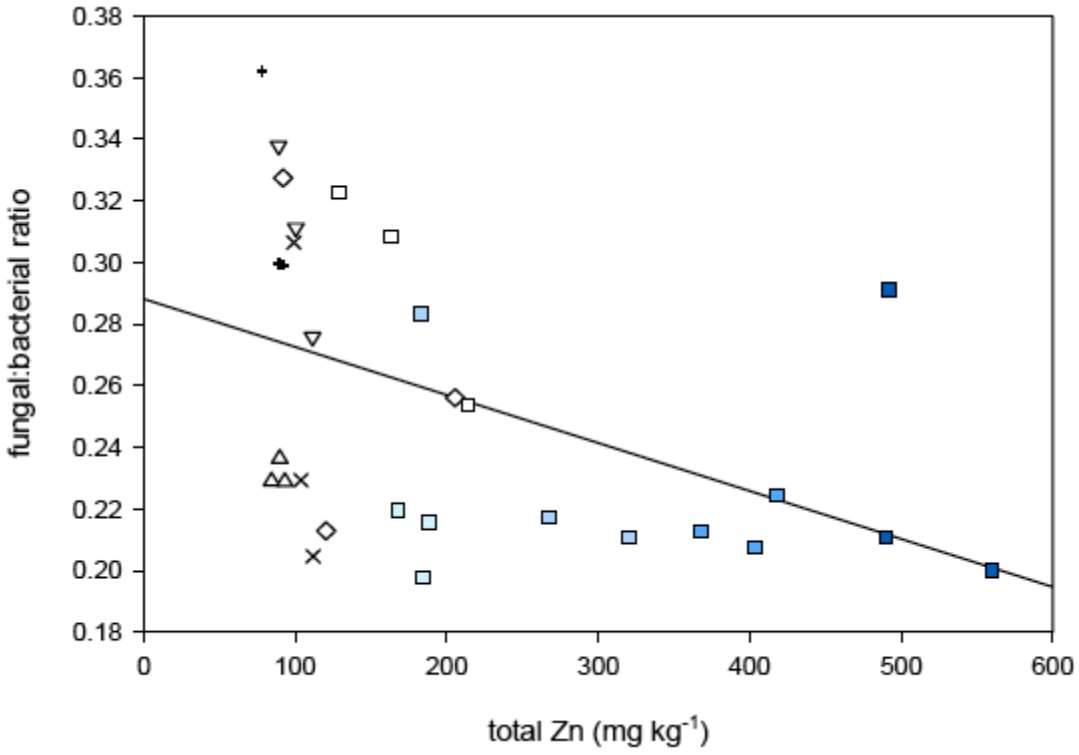

Figure 2a **.** Correlation of total Cu and Zn with PLFA for Auchincruive LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◇), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (○), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
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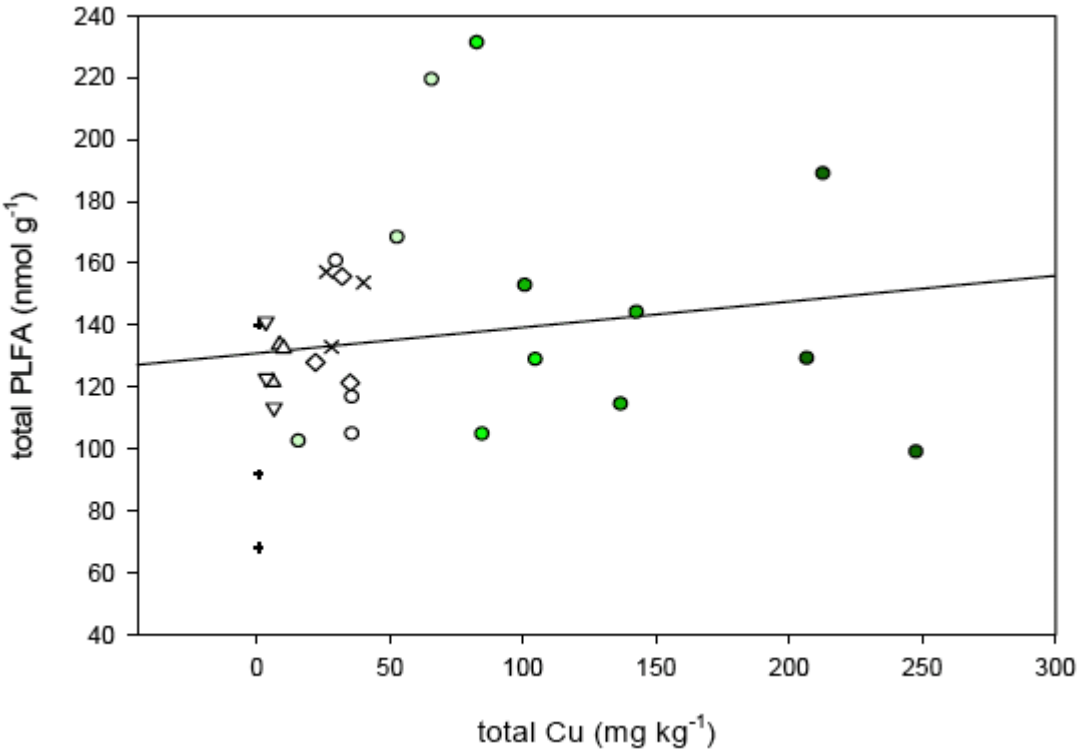

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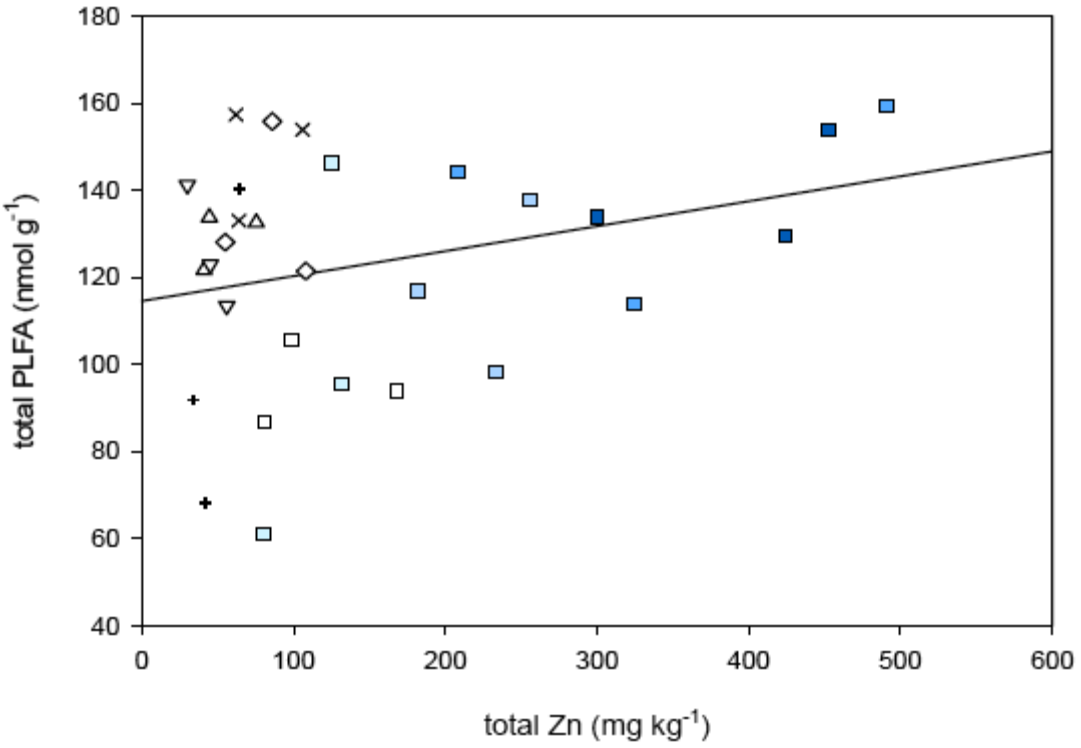

Figure 2b **.** Correlation of total Cu and Zn with fungal PLFA for Auchincruive LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◇), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
metal LTB (△), Zn LTB (□), Zn 150 (■), Zn 200 (■), Zn 250 (■), Zn 450 (■). Each
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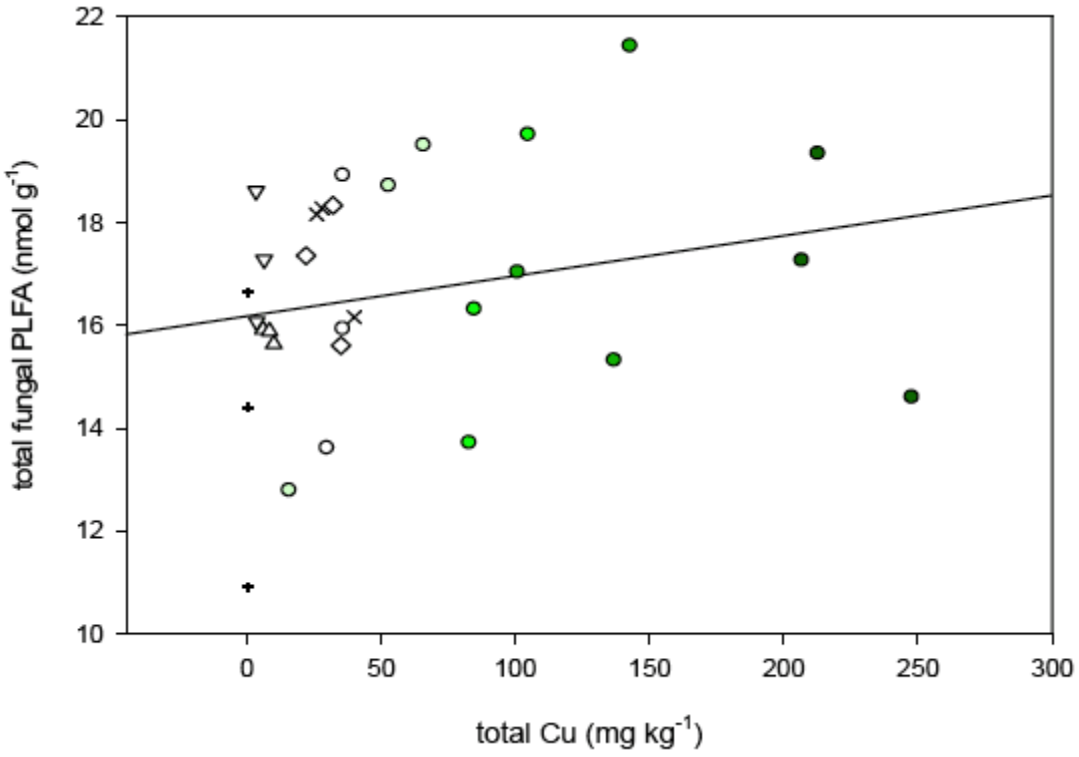
total fungal PLFA (nmol g-1)
total Cu (mg kg-1)
22
20
18
16
14
12
10
0
50
100
150
200
250
300

ii)

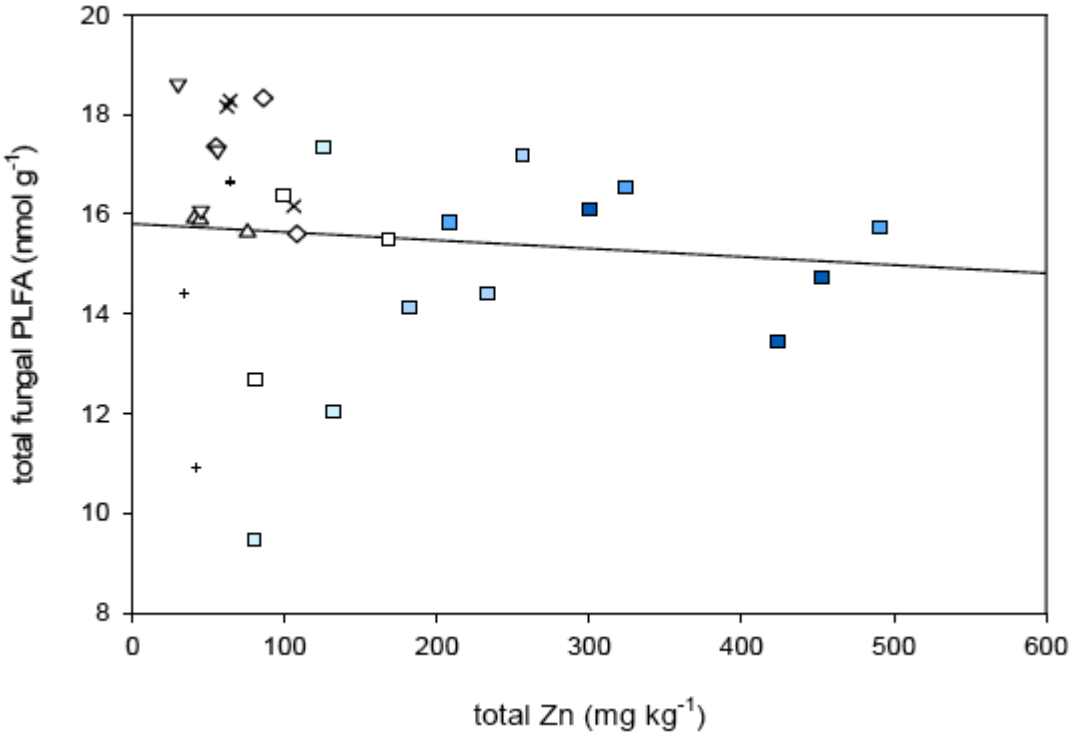
total fungal PLFA (nmol g-1)
total Zn (mg kg-1)
20
18
16
14
12
10
8
0
100
200
300
400
500
600

Figure 2c. Correlation of total Cu and Zn with NLFA C16:1?5c lipid (indicative of AM fungi) for Auchincruive LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◇), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
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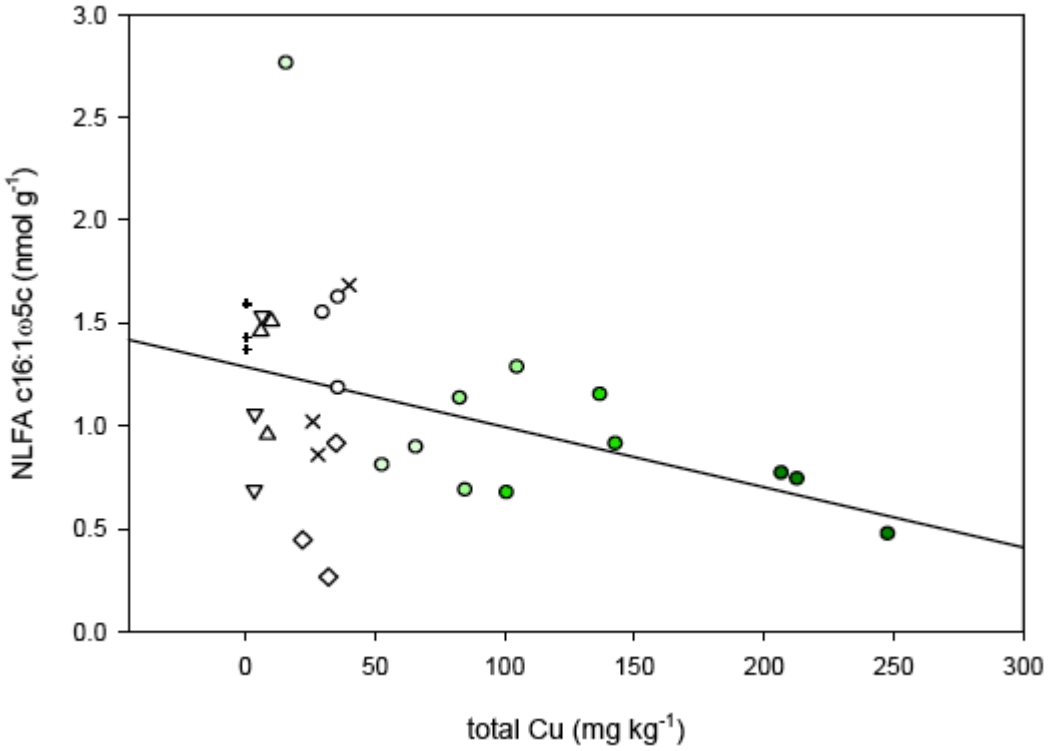

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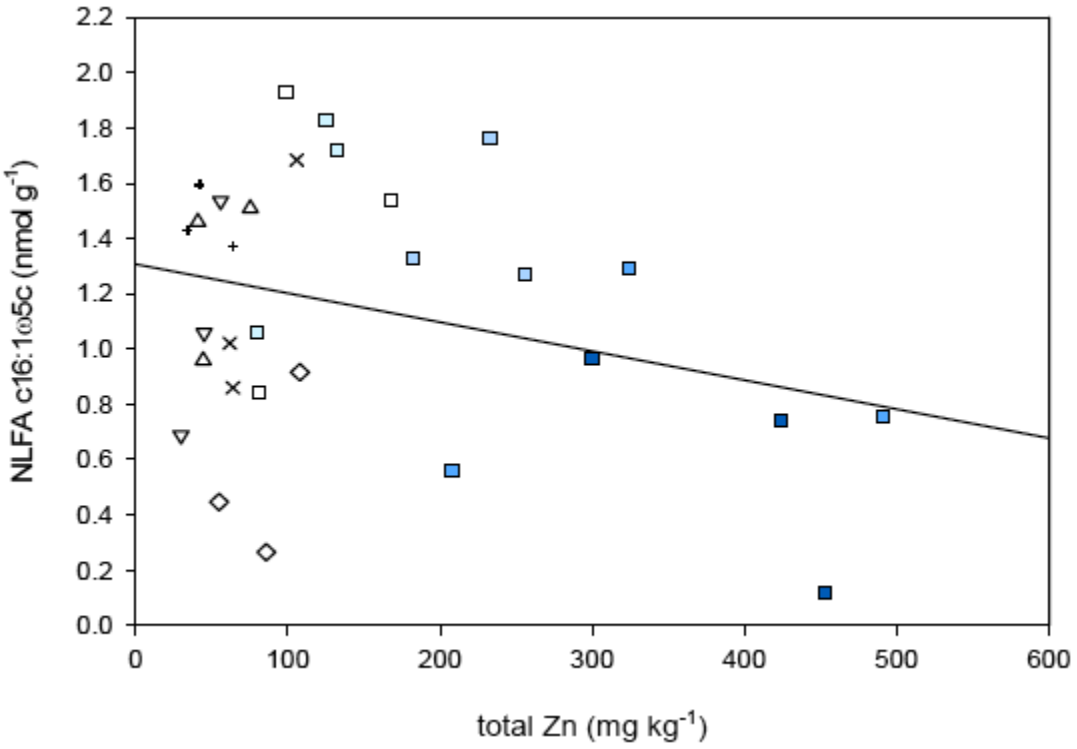

Figure 2d **.** Correlation of total Cu and Zn with bacterial PLFA for Auchincruive LTSE on selected soil treatments **.** i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◈), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
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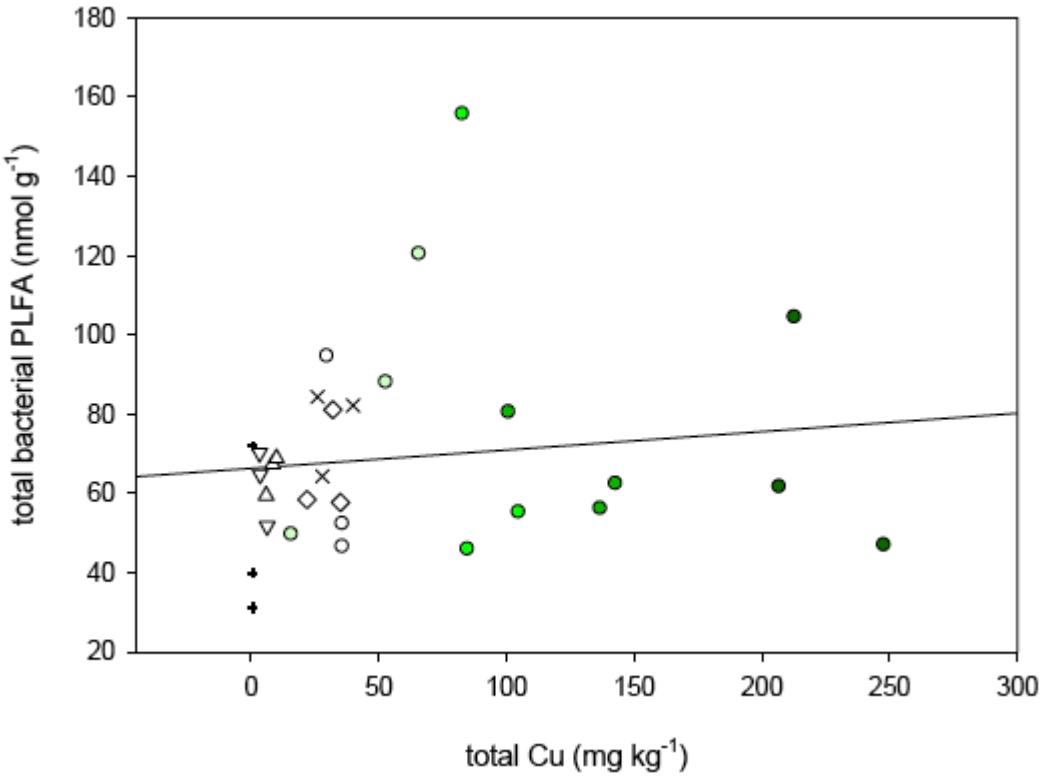
total bacterial PLFA (nmol g-1)
total Cu (mg kg-1)
180
160
140
120
100
80
60
40
20
0
50
100
150
200
250
300

ii)

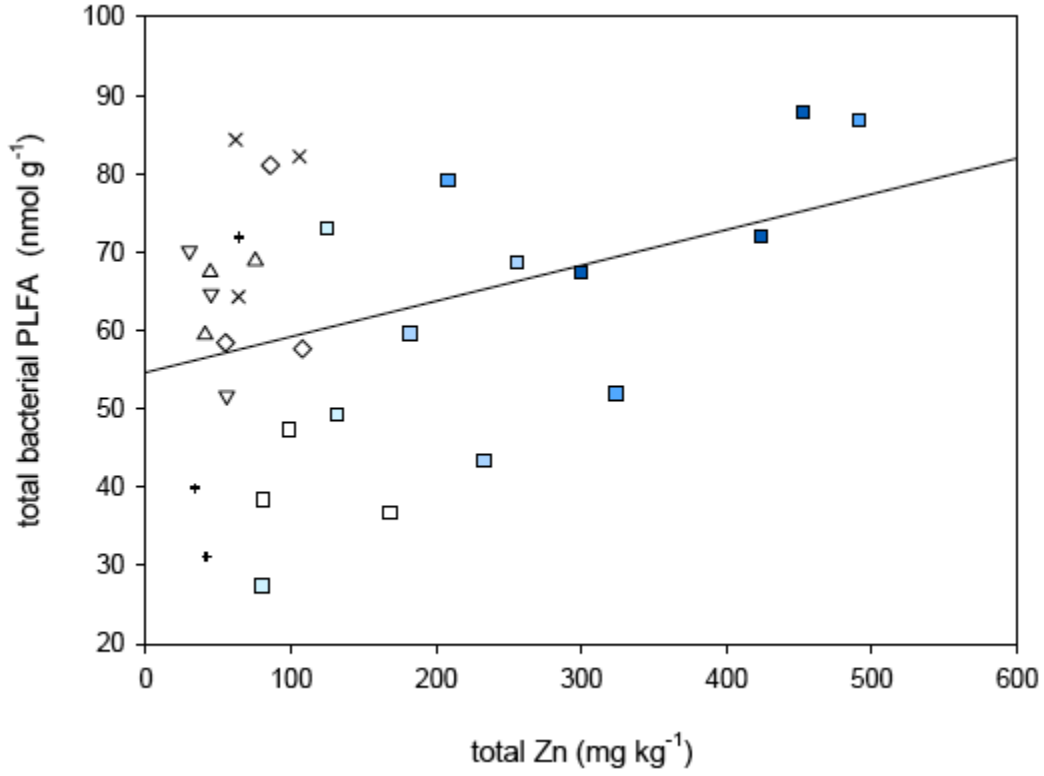
total bacterial PLFA (nmol g-1)
total Zn (mg kg-1)
100
90
80
70
60
50
40
30
20
0
100
200
300
400
500
600

Figure 2e. Correlation of total Cu and Zn with fungal:bacterial ratio for Auchincruive LTSE on selected soil treatments . i) Cu treatments with controls and undigested and digested controls; ii) Zn treatments with controls and undigested and digested controls.

Legend: Control (+), Undigested (◈), Undigested Long Term Build up (LTB) (▽), C
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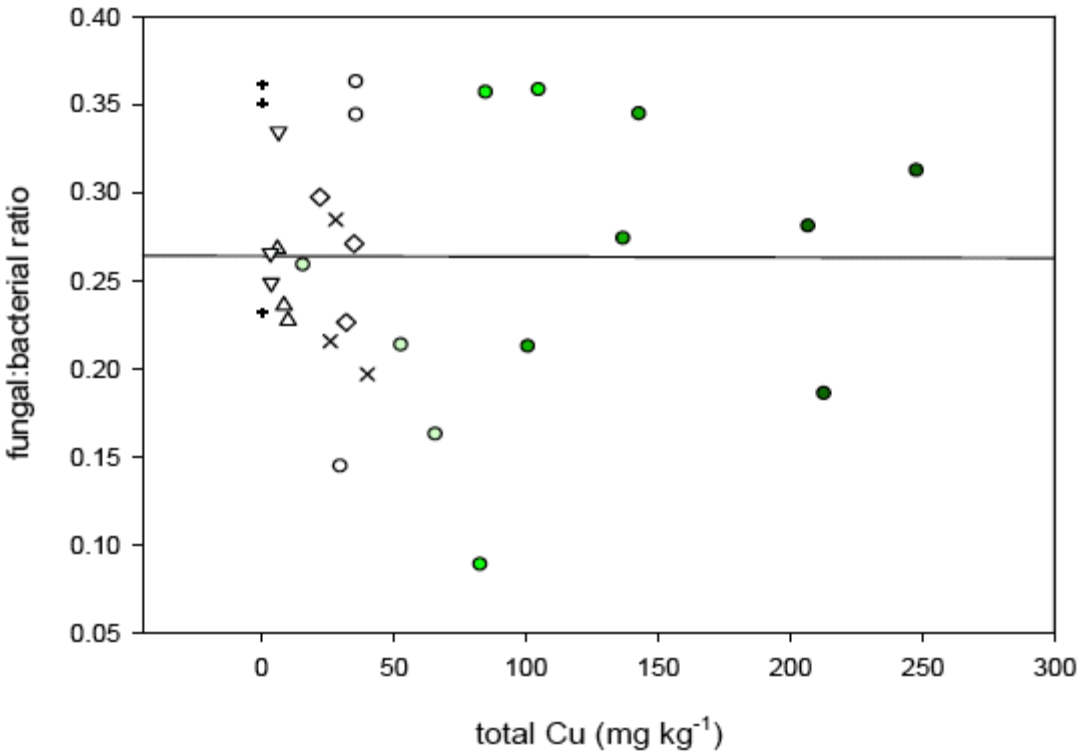

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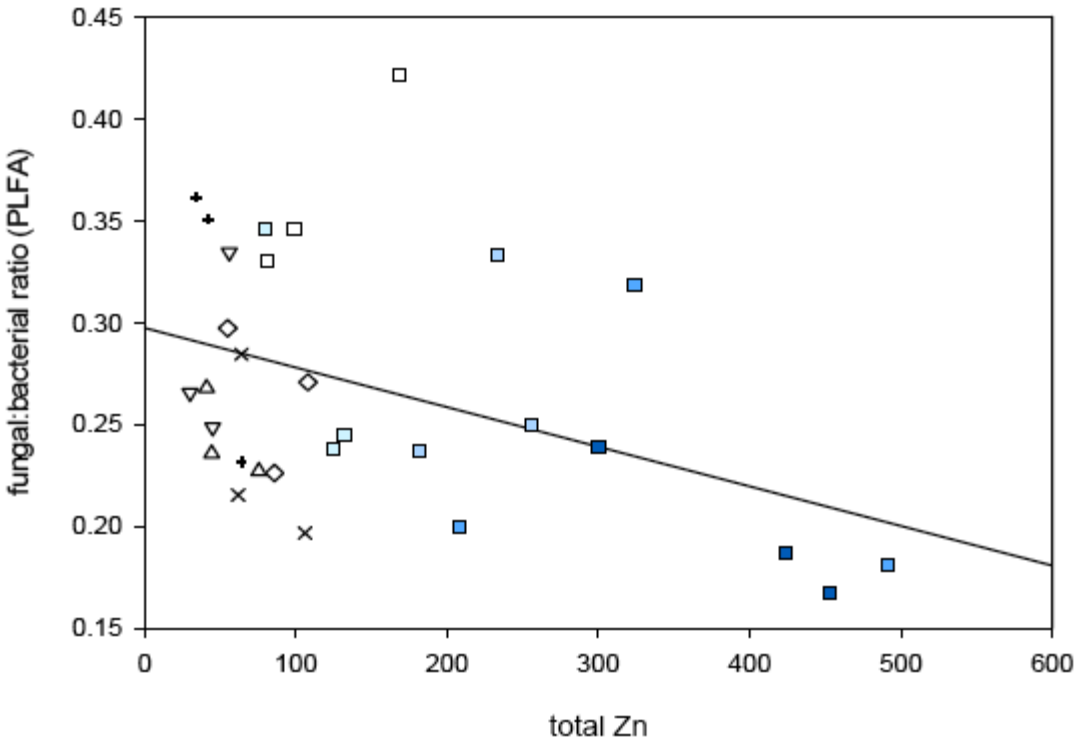
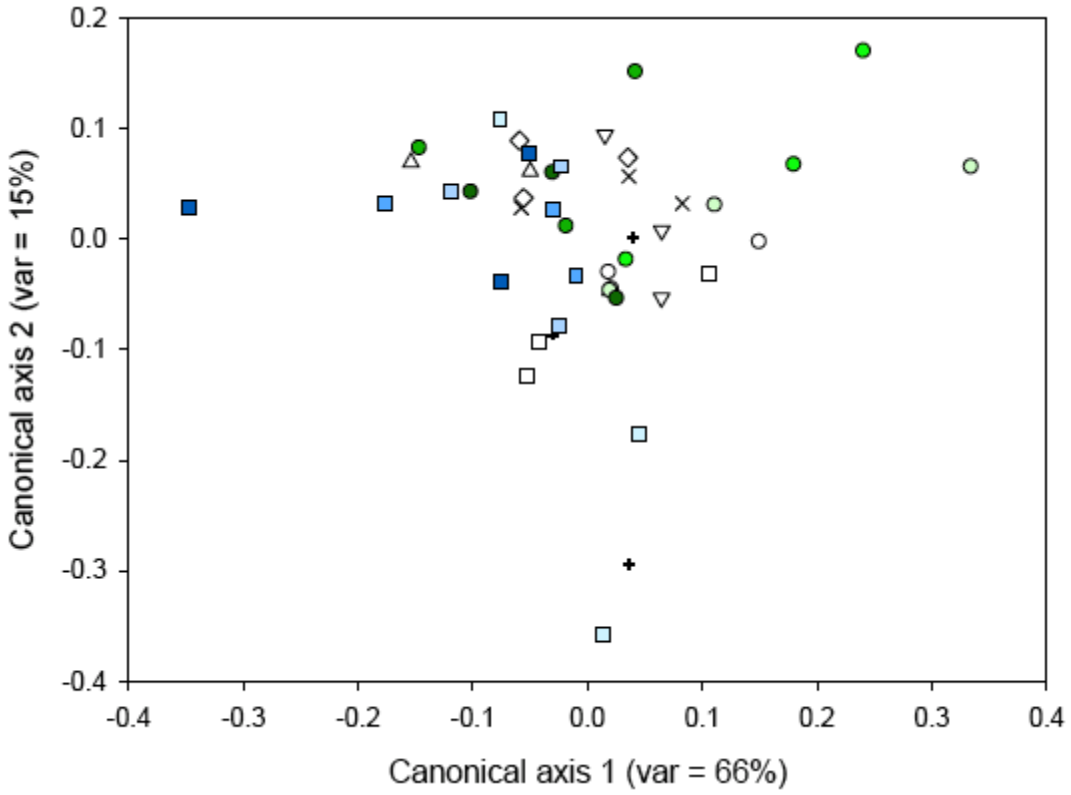


Figure 3. Canonical analysis of PLFA from a) Auchincruive and b) Hartwood

Legend: Control (+), Undigested (◈), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
metal LTB (△), Zn LTB (□), Zn 150 (■), Zn 200 (■), Zn 250 (■), Zn 450 (■). Each
replicate block is shown as a separate symbol.

a)


Canonical axis 2 (var = 15%)
Canonical axis 1 (var = 66%)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4

b)

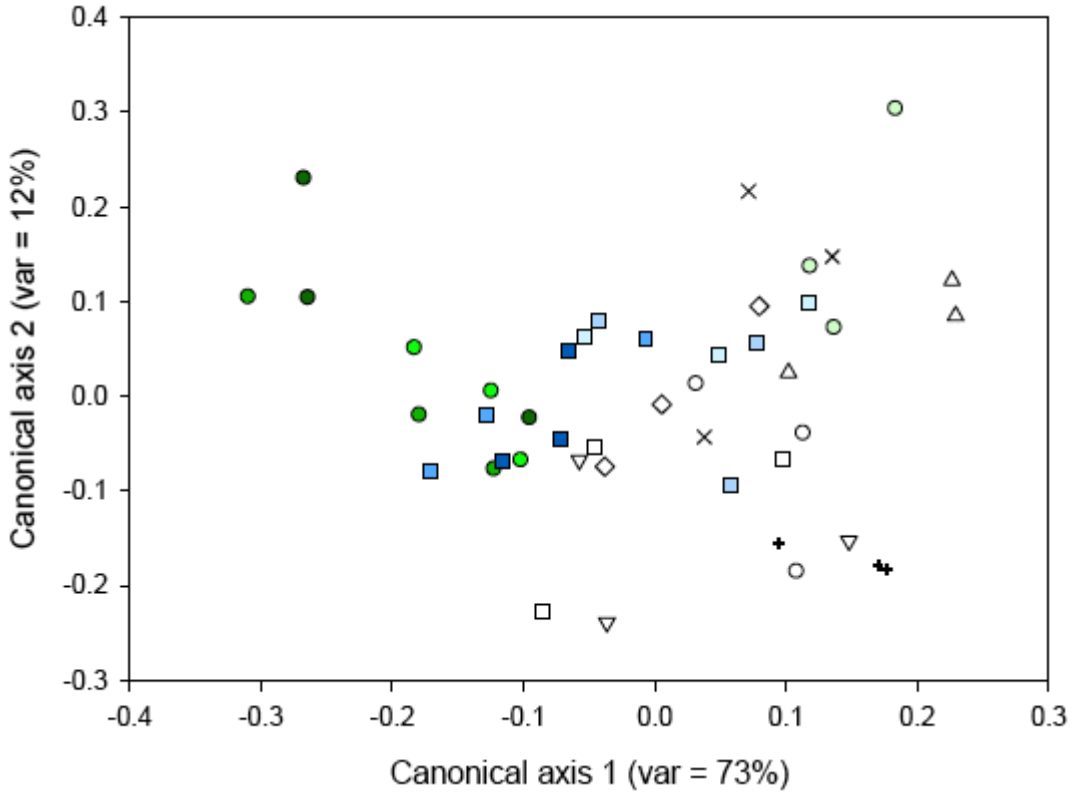

Canonical axis 2 (var = 12%)
Canonical axis 1 (var = 73%)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

Table 2a. Correlation coefficients (r) of Cu (50, 100, 150, 200) treated soils controls, undigested and digested controls with different lipid markers for Hartwood and Auchincruive LTSE plots. Correlations significant at =5% are underlined.

Lipid marker	Hartwood	Auchincruive
Total PLFA (microbial biomass)	-0.128	0.164
Fungal PLFA	-0.495	0.234
NLFA_C16?5c (AM fungi)	-0.532	-0.405
Bacterial PLFA	0.024	0.125
Fungal:bacterial ratio	-0.648	-0.033

Table 2b. Correlation coefficients (r) of Zn (150, 250, 350, 450) treated soils, controls, undigested and digested controls with different lipid markers for Hartwood and Auchincruive LTSE plots. Correlations significant at =5% are underlined.

Lipid marker	Hartwood	Auchincruive
Total PLFA (microbial biomass)	-0.062	0.288
Fungal PLFA	-0.314	-0.100
NLFA_C16?5c (AM fungi)	-0.487	-0.290
Bacterial PLFA	0.019	0.355
Fungal:bacterial ratio	-0.456	-0.407

Table 3. Correlation coefficients (r) of %N with different lipid markers for Hartwood and Auchincruive LTSE plots for all soils used. Correlations significant at =5% are underlined.

Lipid marker	Hartwood	Auchincruive
Total PLFA (microbial biomass)	-0.282	0.346
Fungal PLFA	0.093	0.272
NLFA_C16?5c (AM fungi)	-0.255	-0.174
Bacterial PLFA	0.331	0.301
Fungal:bacterial ratio	-0.343	-0.281

Substrate utilisation tests

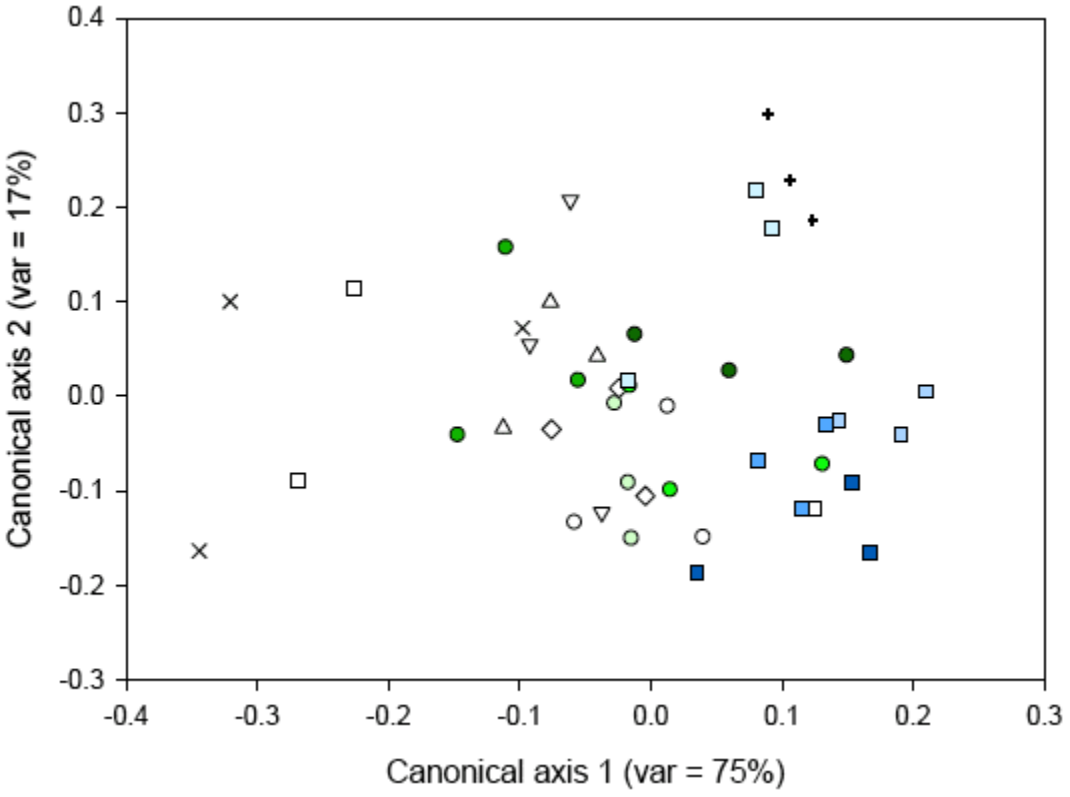
Substrate utilisation of 15 carbon (C) sources was tested using the MicroResp™ system (Campbell *et al*., 2003). The C sources used were chosen to reflect easily metabolisable compounds derived from root exudates and monomers of more complex polymers that might be released during decomposition and hydrolysis. There were no significant differences between the soil treatments of either soils (data not shown). Highest respiration rate was found with a-ketoglutarate which is used in the citric acid cycle and a precursor for glutamate and several other amino acids (aminoadipic acid metabolic pathway) indicating a link with N metabolism (data not shown).

Analysis by multivariate techniques showed some separation between treatments for both soils (Fig. 4) but this was more evident in the soils from Hartwood (Fig. 4a) than from Auchincruive (Fig. 4b).

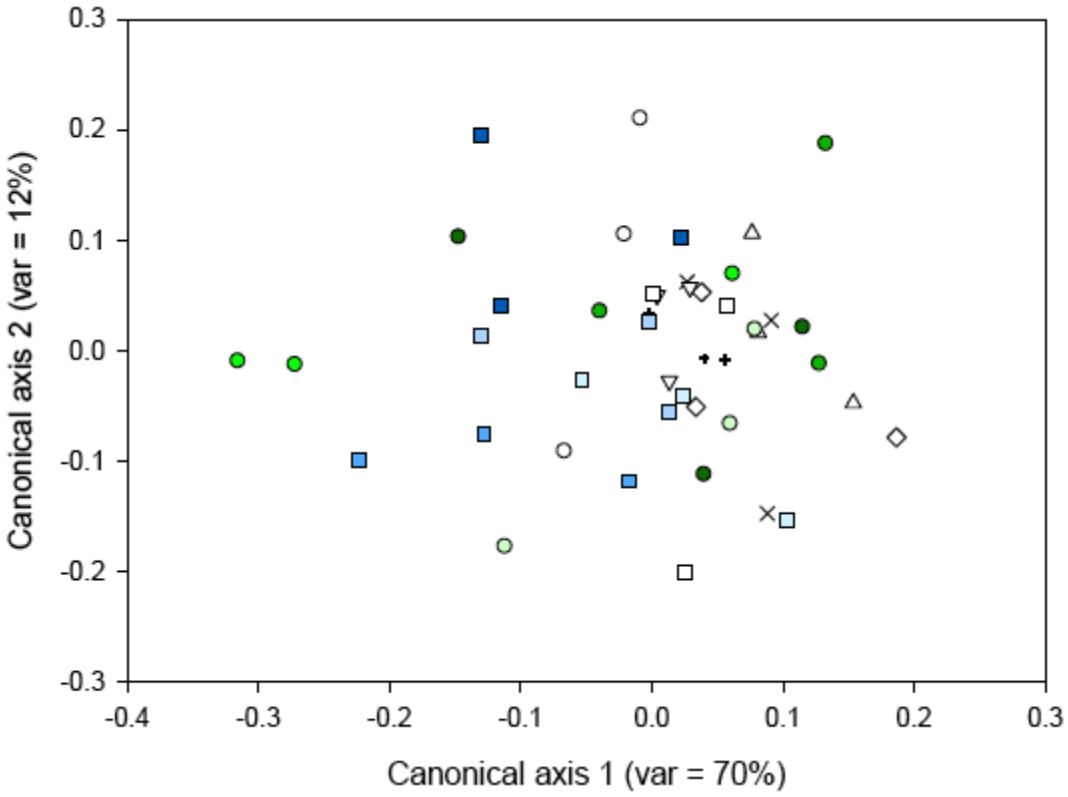
Figure 4. Canonical multivariate analysis of MicroResp™ physiological profiles (C source metabolism)

Legend: Control (+), Undigested (◇), Undigested Long Term Build up (LTB) (▽), C
(○), Cu 50 (●), Cu 100 (●), Cu 150 (●), Cu 200 (●), Digested low metal (X), Digest
metal LTB (△), Zn LTB (□), Zn 150 (■), Zn 200 (■), Zn 250 (■), Zn 450 (■). Each
replicate block is shown as a separate symbol.

a) Hartwood



b) Auchincruive



C-catabolism

The degradation (catabolism) of a range of xenobiotics over 75 days was tested using the ^{14}C MicroResp™ system (Campbell *et al*., 2003) on control soils, Cu 200 and Zn 450 rich sludge treatments and LTB sludge treatments from both sites.

Typical degradation kinetics were found for carbon sources in the soils tested. The percent mineralised for different C sources ranged from ~1% (e.g. isoproturon) to ~30% (glucose).

The % total mineralization of 2,4-dichlorophenoxyacetic acid (2,4-D) was higher for Hartwood than for Auchincruive. The total mineralization of the herbicides atrazine, simazine and isoproturon was lower than that of glucose and 2,4-D, pyrene and glyphosate in both soils. Soils that had been treated with sludge had slightly faster degradation rates for pyrene and water-soluble ryegrass-extract than in control soils but these differences were not significant. There were no significant effects of metal rich sludge found on the catabolism of any of these compounds (Fig. 5a, b). Similarly the degradation of ^{14}C-labeled ryegrass showed no impact of any treatment at either site with rates comparable between the two soils (Fig. 6a, b).

DT_{50} values (the number of days at which 50% of the substrate under test is degraded) were estimated from the level of CO_2 produced and ranged from around 20 days (glucose) to 500 days (isoproturon) (Table 4) and assumes a 50% efficiency of C turnover. Significant differences were found in the Hartwood soil using glyphosate between the control soil and the Zn LTB. DT_{50}

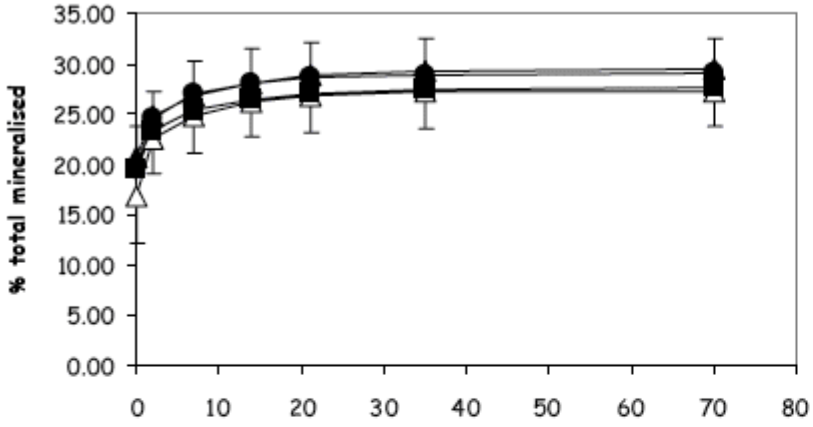
for 2,4-D was longer in soil from Auchincruive (but not significantly due to block I replicate) than in the Hartwood soil.

Figure 5. Percent total mineralisation of ^{14}C labeled substrates after addition of ^{14}C labeled glucose (positive control) and seven ^{14}C labeled xenobiotics to soils from LTSE at a) Auchincruive and b) Hartwood for soils receiving either no sludge,

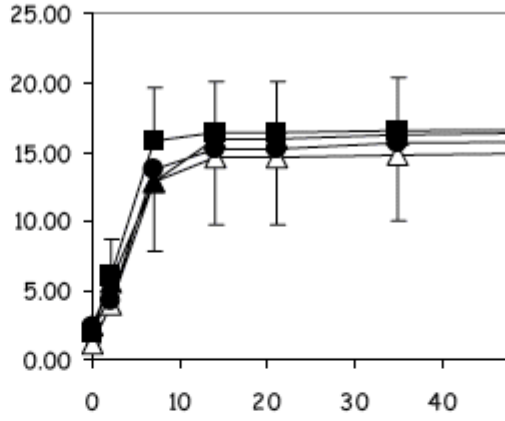
Control (-▵-); or sludge rich in Cu 200 (-●-); or Zn 450 (-■-);
or long term build up sludge (-▲-) Error bars are +/- std. dev.
For clarity only those error bars on the outer lines are shown.

5a)

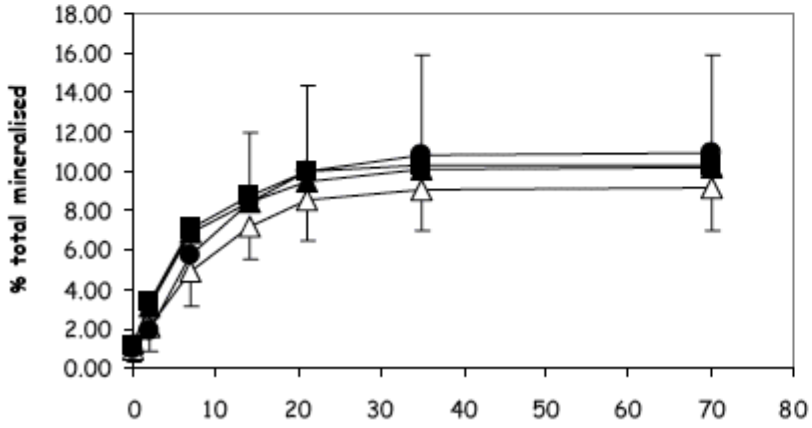
Degradation of glucose



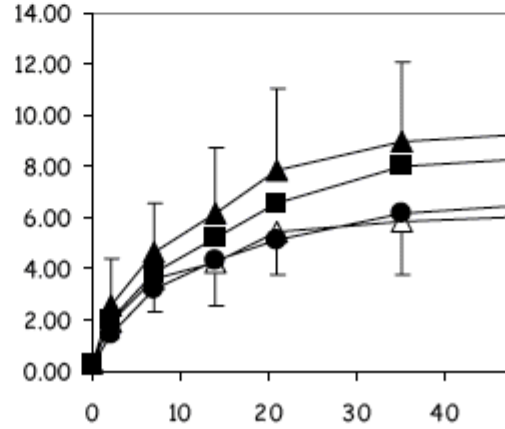
Degradation of salicylic



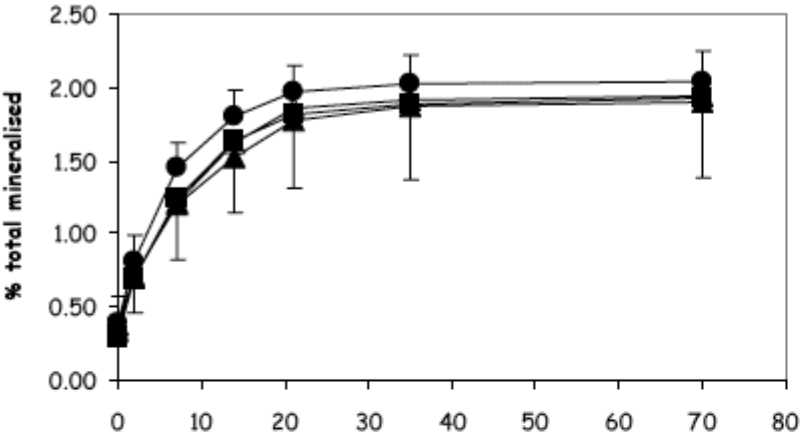
Degradation of 2,4-D



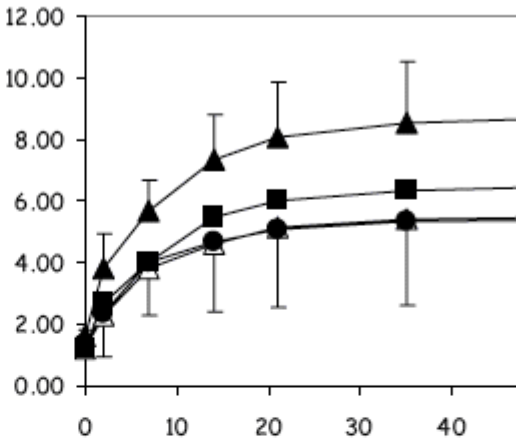
Degradation of pyre



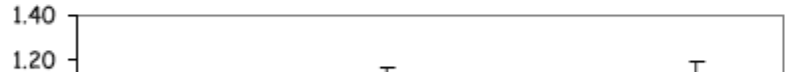
Degradation of atrazine



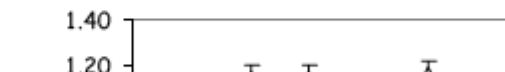
Degradation of glyphos



Degradation of isoproturon

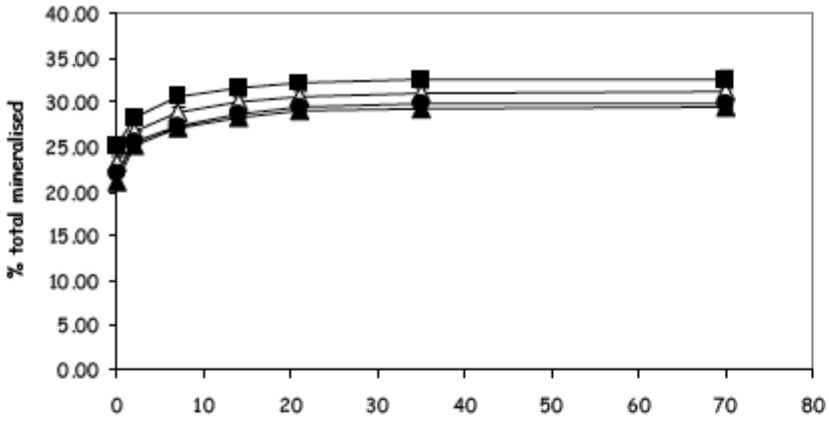


Degradation of simaz

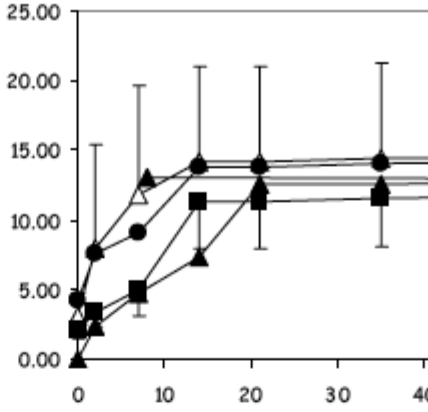


5b)

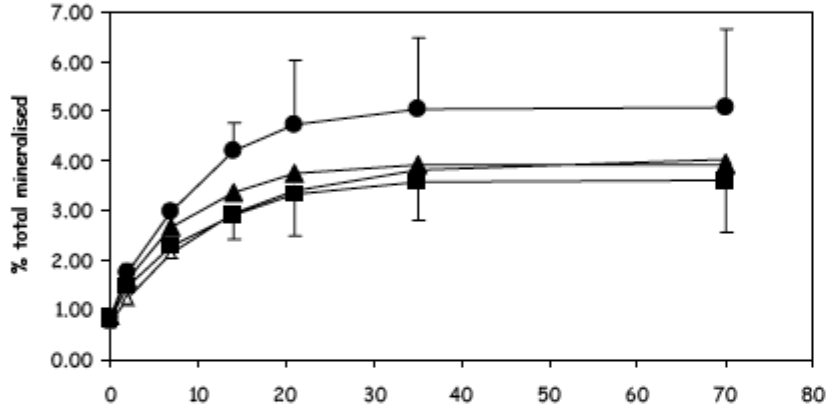
Degradation of glucose



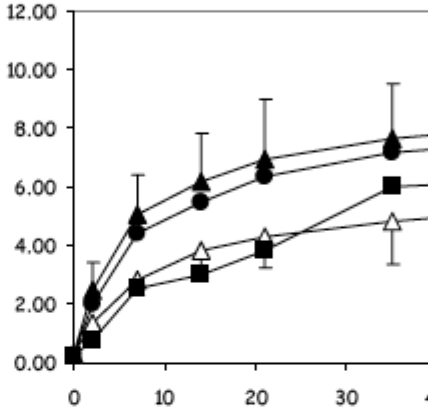
Degradation of sali



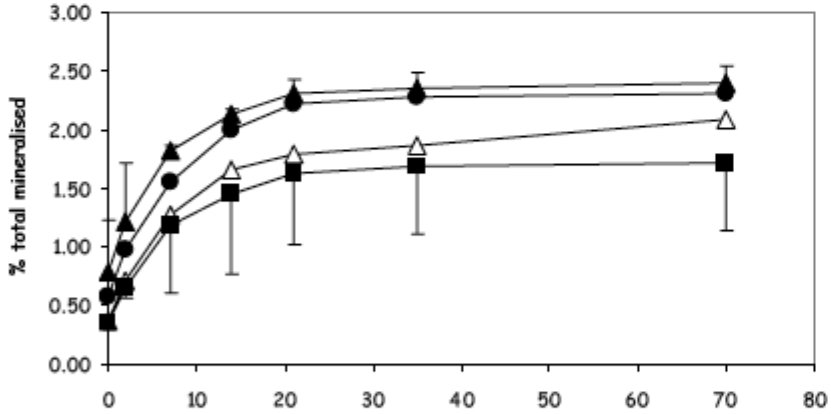
Degradation of 2,4-D



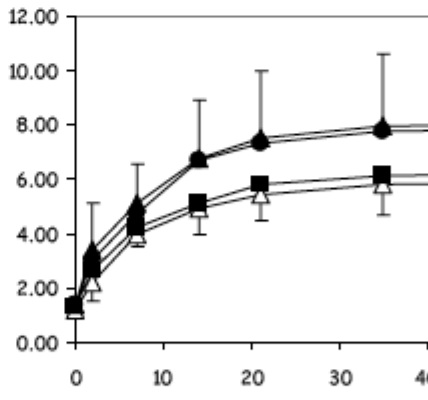
Degradation of



Degradation of atrazine



Degradation of gly



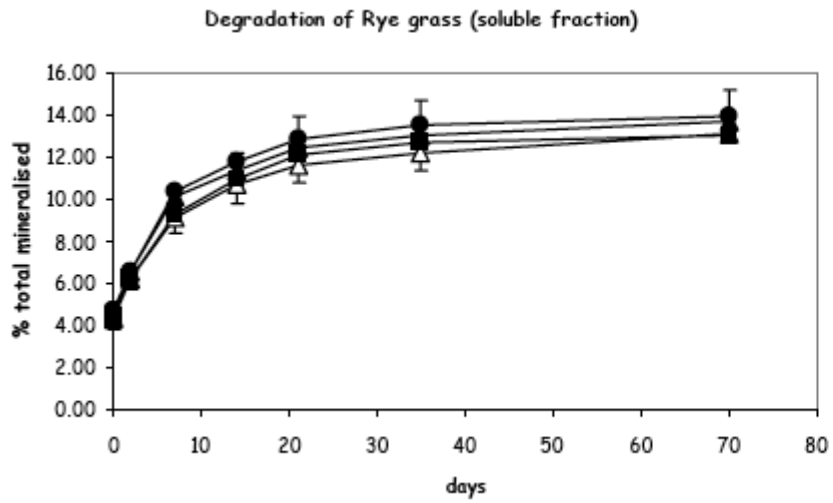
Degradation of isoproturon

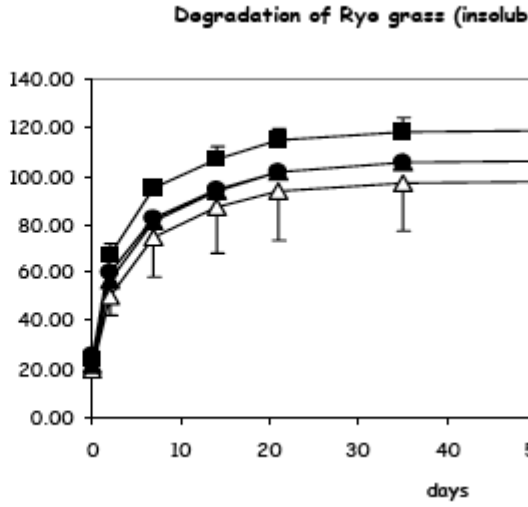
Degradation of

Figure 6. Percent total mineralisation of ^{14}C labeled ryegrass or water soluble extract of ryegrass added to soils from LTSE at a) Auchincruive and b) Hartwood

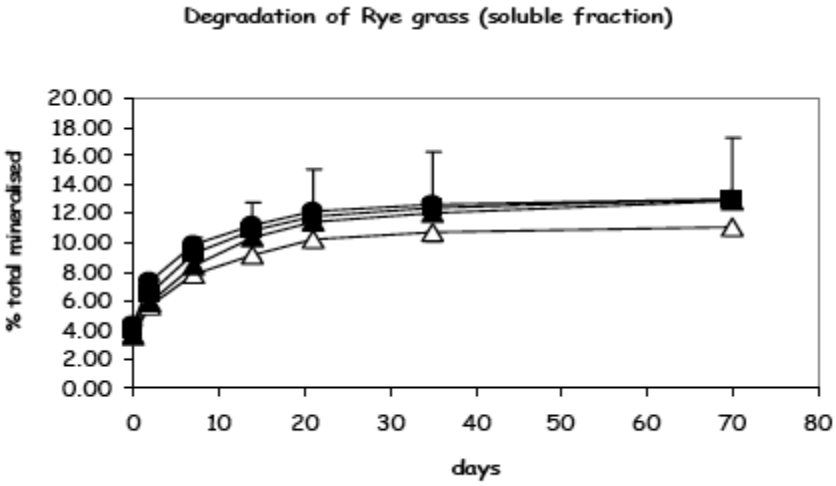
Control (-▵-); or sludge rich in Cu 200 (-●-); or Zn 450 (-■-);
or long term build up sludge (-▲-) Error bars are +/- std. dev.
For clarity only those error bars on the outer lines are shown.

a) Auchincruive





b) Hartwood



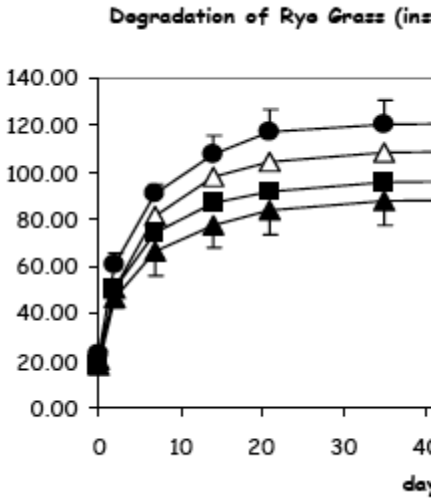


Table 4. Summary of DT_{50} (days) for ^{14}C labelled xenobiotics added to sludge treated soils. Average values from block replicates. Different letters for each carbon substrate denotes values which are significantly ($P<0.05$) different. LTB - Long Term Build up

Soil	Treatment	2,4-D	atrazine	glyphosate	Isoproturon	pyrene	simazine	glucose	salicylic acid
Auchincruive	Control	79 [a]	138 [a]	53 [ab]	516 [a]	50 [a]	220 [a]	20 [a]	21 [a]
	Cu 200	66 [a]	154 [a]	50 [ab]	353 [a]	29 [a]	281 [a]	21 [a]	25 [a]
	Zn LTB	91 [a]	154 [a]	52 [ab]	435 [a]	41 [a]	251 [a]	22 [a]	21 [a]
	Zn 450	81 [a]	177 [a]	57 [ab]	490 [a]	27 [a]	269 [a]	20 [a]	20 [a]
Hartwood	Control	32 [a]	156 [a]	71 [b]	479 [a]	50 [a]	246 [a]	20 [a]	21 [a]
	Cu 200	34 [a]	157 [a]	64 [ab]	363 [a]	31 [a]	281 [a]	21 [a]	24 [a]
	Zn LTB	28 [a]	142 [a]	47 [a]	475 [a]	29 [a]	251 [a]	20 [a]	20 [a]
	Zn 450	28 [a]	150 [a]	70 [ab]	341 [a]	27 [a]	257 [a]	22 [a]	24 [a]
	F.pr	0.024	0.682	0.016	0.789	0.24	0.684	0.811	0.988
	LSD	44.8	41.4	14.4	282.3	24.4	73.6	2.91	13.82
DT_{50} literature value		8	19-120	10-16	11-35; 70	25-1840	6-40	-	-
Reference		Linder *et al*., 1994	Sarmah *et al* ., 2009	Kools *et al*., 2005	Navarro *et al* ., 2009; Rodríguez-Cruz *et al*., (2006)	Thiele-Bruhn & Brümmer (2005)	Hixson *et al*., 2009		

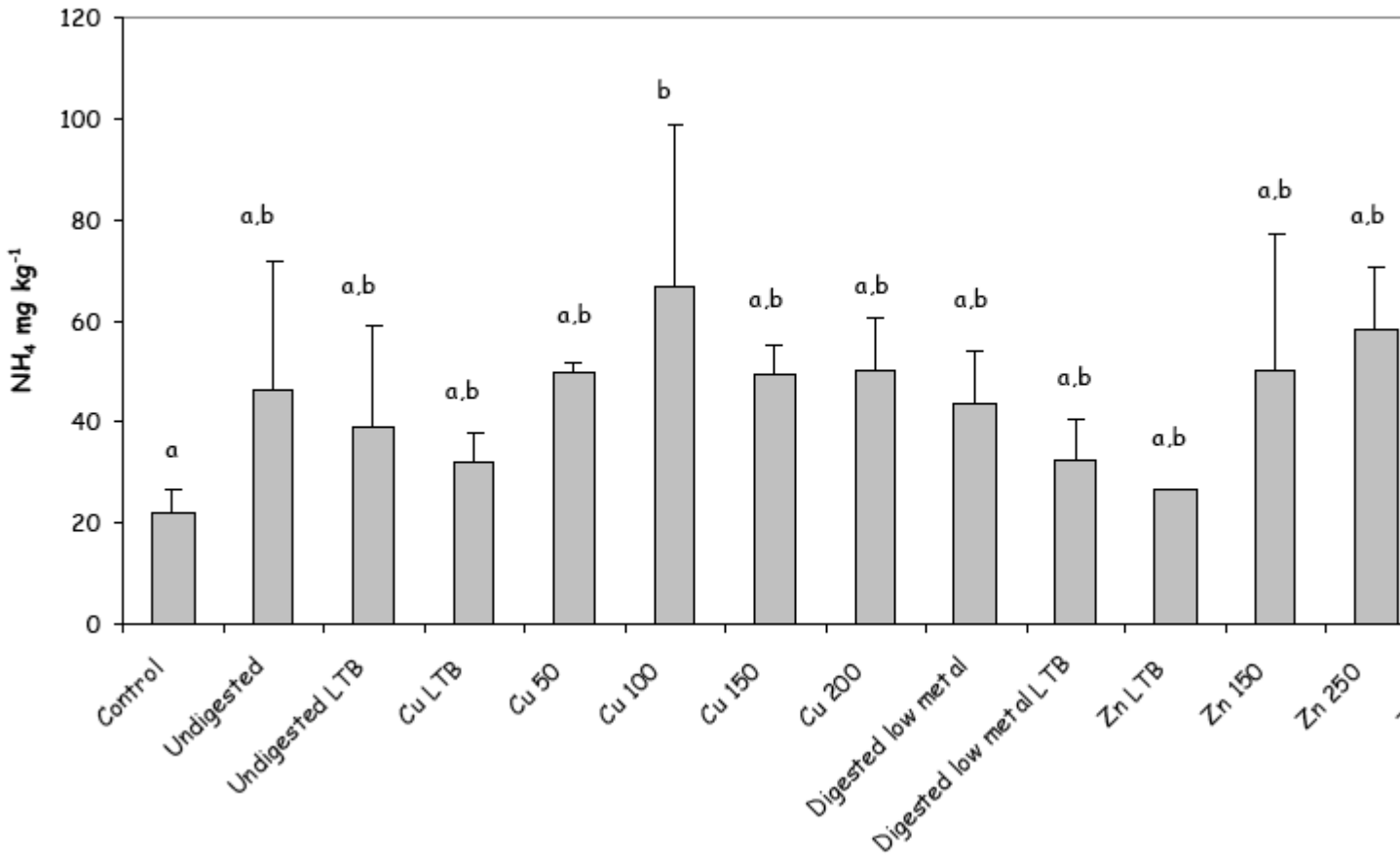
N-mineralisation and potential nitrification

N-mineralisation (as NH_4^+-N) was measured in both Hartwood and Auchincruive soils as part of the main LTSE programme. There was a weakly significant (P=10%) difference between the control and Cu 100 with Auchincruive soil (Fig. 7a). With Hartwood soils, significant differences were found between undigested, Cu LTB and Zn LTB treatments (Fig. 7b). No significant correlation was found between N mineralization and Cu or Zn levels.

Potential nitrification (transformation of ammonium-N to nitrite and nitrate-N) was measured by incubation with $(NH_4)_2SO_4$. Generally the potential nitrification rates were higher in Auchincruive soil than at Hartwood. There was no significant difference between the rate of nitrite production of either soil (Fig. 8 a, b) or of total organic N (data not shown). Nitrate values (data not shown) were measured but were generally found to be unreliable with some of the control (frozen) values higher than the incubated samples (data not shown).

Figure 7. N-mineralisation (NH_4 mg kg^{-1} soil) from soils taken at a) Auchincruive (F. pr = 0.059, l.s.d. = 24.9) and b) Hartwood (F. pr = 0.208, l.s.d. =123.0) in 2008 as part of the LTSE experimental protocol. Error bars are standard deviations. LTB- Long Term Build up

a) Auchincruive


120
100
80
60
40
20
0
NH4 mg kg-1
a
a,b
a,b
a,b
a,b
b
a,b
a,b
a,b
a,b
a,b
a,b
a,b
Control
Undigested
Undigested L TB
Cu L TB
Cu 50
Cu 100
Cu 150
Cu 200
Digested low metal
Digested low metal L TB
Zn L TB
Zn 150
Zn 250

b) Hartwood

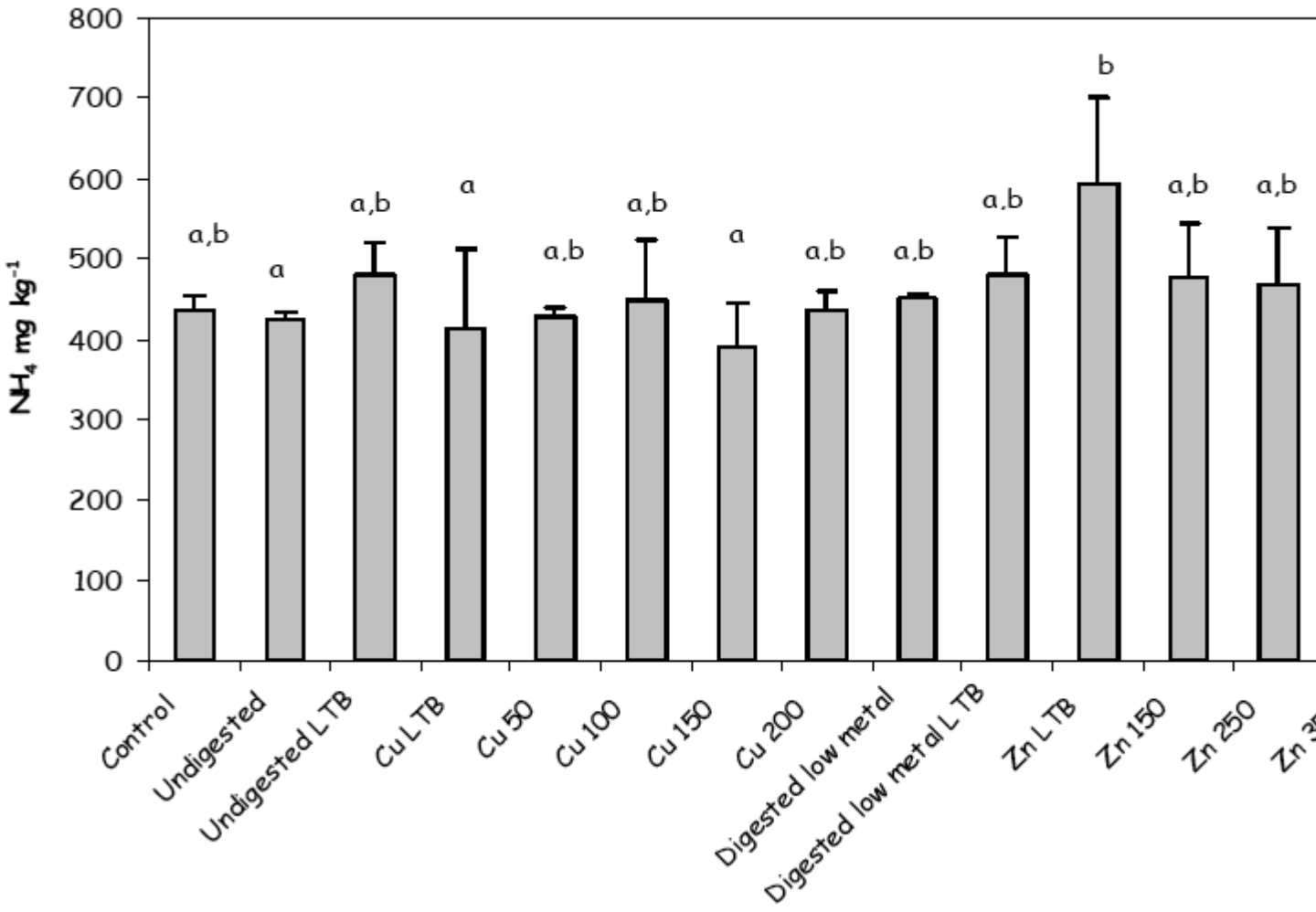
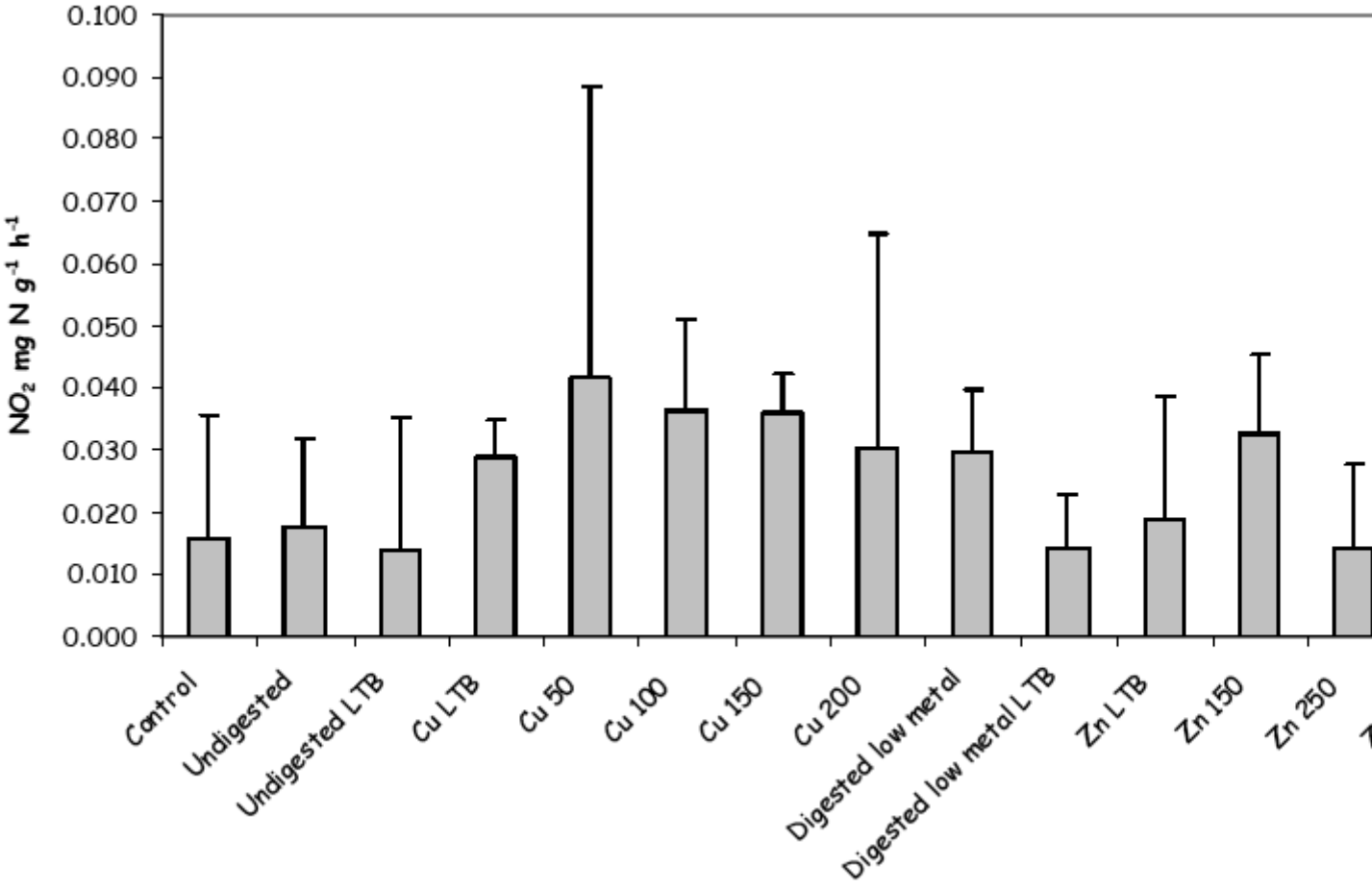
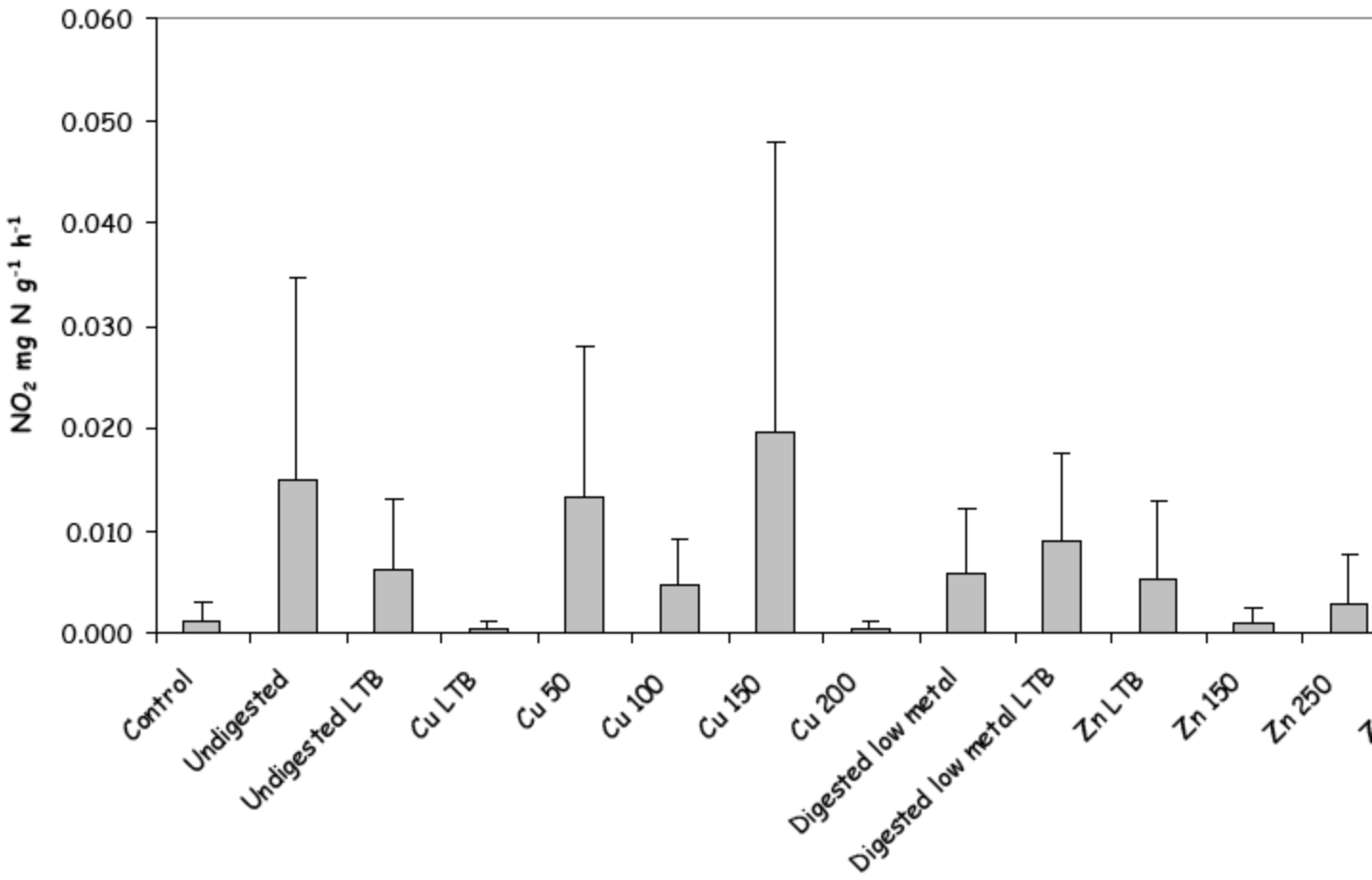


Figure 8. Potential nitrification as measured by production of NO_2^- of a) Auchincruive soil (F. pr = 0.579, L.s.d.=0.036) and b) Hartwood (F. pr. =0.698, L.s.d.= 0.021). Error bars are standard deviations. LTB- Long Term Build up.

a) Auchincruive


0.100
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
NO_2 mg N g^{-1} h^{-1}
Control
Undigested
Undigested LTB
Cu LTB
Cu 50
Cu 100
Cu 150
Cu 200
Digested low metal
Digested low metal LTB
Zn LTB
Zn 150
Zn 250

b) Hartwood



N-fixation potential and recovery of nodulation of clover.

A microcosm experiment was established to compare different ways of re-introducing Rhizobium into the soils most affected by Zn rich sludge from Hartwood by using either a commercial inoculum applied as a seed coat or by adding soil from control plots (10% (w/w) equivalent dry weight) which had had no sludge added to them.

In pots with no plants and/or no inoculum of either soil or commercial rhizobia inoculum, the soil population of rhizobia (as MPN) did not increase over the 147 day period (Fig. 9, Table 5). Clover grown on its own in unamended control soil significantly increased the rhizobia MPN progressively over the 147 days. Soil from Zn 350 plots without any inoculation or clover plants showed no real signs of recovery over the 147 day period. With the addition of clover to the Zn 350 soil, there was a small increase in the MPN but not to the levels seen in the control (unamended) soil. Rhizobium MPN were further increased by addition of 10% (w/w) inoculum of control soil and also by the commercial inoculum of Rhizobium. The Rhizobium population in the inoculated Zn 350 soils was generally sustained over the 147 day period but was less than in the control soil.

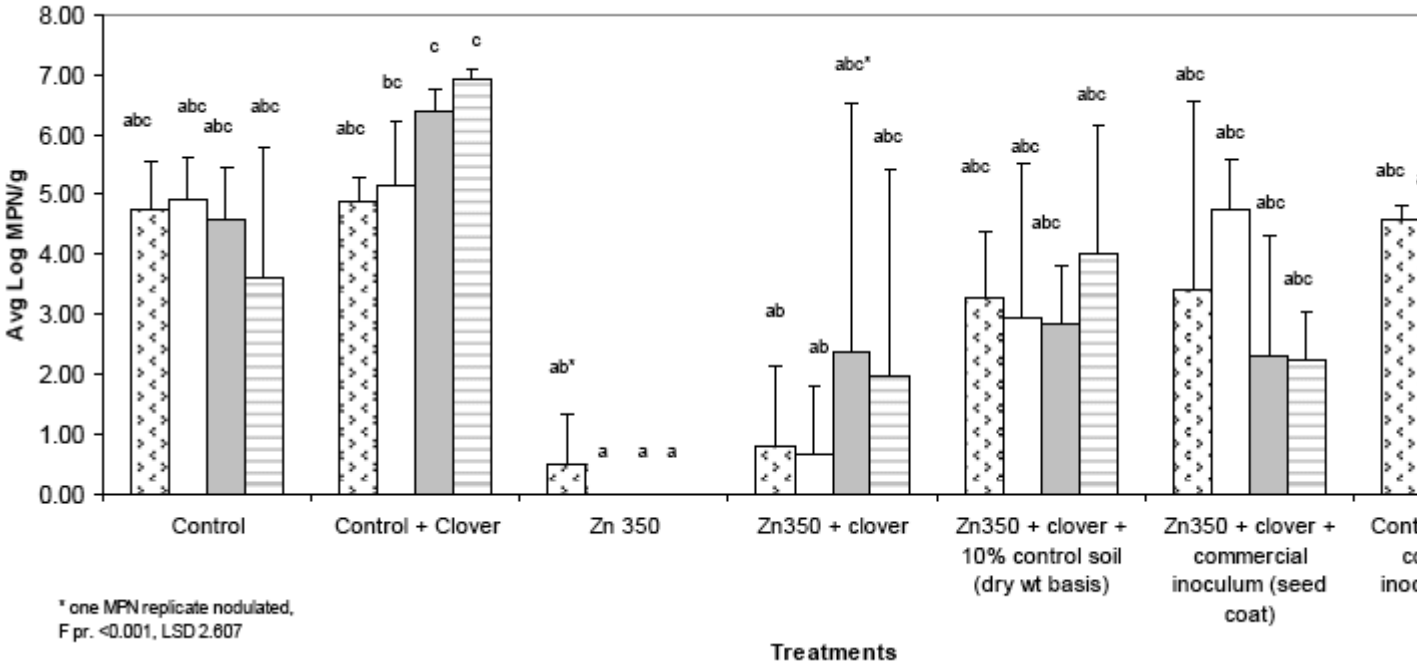


Figure 9. Most probable number of Rhizobium in soil from different plots at Hartwood planted with or without clover and inoculated with either control soil or a commercial Rhizobium inoculum (as a seed coat) or both over four harvest dates. Error bars are standard deviations. Different letters denote significant differences within that treatment. (F pr. = <0.001, L.S.D. = 2.607)

Rates of potential N fixation were measured from a selection of MPN tubes (Table 6). MPN tubes with no nodulated clover plants showed no N-fixation and nether did soil (no plant) on its own from any of the treatments (data not shown). There was no significant difference in the ethylene production rates from harvest dates I or II. At harvest date III, no ethylene production was found with the Zn 350, Zn 350 & clover, Zn 350 & clover & 10% soil inoculum.

Table 5. Summary of main effects of pot experiment using Hartwood soil

Treatment	Recovery of rhizobia
No remedial treatment	No
+clover	slight
+clover+ 10% soil inoculum	Yes
+clover + commercial inoculum	Yes

Table 6. Rate of ethylene production from acetylene (µmoles C_2H_2 h^{-1}) for different pot treatments with Hartwood soil receiving different rhizobia inocula over three harvests. Rates are from 10^{-0}MPN soil dilution and are normalized against the rate from a blank (MPN clover plant, no inoculum) sample.

Treatment	Day 0		Day 49		Day 91		Day 147	
	µmoles C_2H_2 h^{-1}	µmoles C_2H_2 h^{-1} nodule $^{-1}$	µmoles C_2H_2 h^{-1}	µmoles C_2H_2 h^{-1} nodule $^{-1}$	µmoles C_2H_2 h^{-1}	µmoles C_2H_2 h^{-1} nodule $^{-1}$	µmoles C_2H_2 h^{-1}	µmoles C_2H_2 h^{-1} nodule $^{-1}$
Control	1.309	0.145	0.078	0.016	0.819	0.153	0.770	0.192
Control + clover	1.589	0.164	1.061	0.114	1.730	0.144	0.491	0.098
Zn 350	1.120	0.560	0.009	0.000	0.002	0.000	0.001	0.000
Zn 350 + clover	0.001	0.000	0.011	0.000	0.000	0.000	0.618	0.143
Zn 350+ clover+ 10% soil inoculum	0.963	0.482	0.335	0.077	0.003	0.002	0.299	0.224
Control + clover + commercial Inoculum	0.986	0.247	0.894	0.107	1.324	0.147	0.631	0.126
Zn 350 + clover + commercial Inoculum	0.087	0.012	0.823	0.124	1.424	0.267	0.015	0.000
F. pr.	0.535		0.149		0.025		0.21	
LSD	1.942		1.000		1.228		0.7265	

Table 7. Summary of main significant effects of Cu and Zn in Hartwood and Auchincruive soils for different major groups of microorganisms and different functional tests.

	Hartwood		Auchincruive	
	Cu	Zn	Cu	Zn
Major Groups				
Total PLFA	No	No	No	No
Fungi	No	No	No	No
Bacteria	No	No	No	No
Fungal:bacterial ratio	**Yes**	**Yes**	No	No
AM fungi	**Yes**	**Yes**	**Yes**	**Yes**
Actinobacter	No	No	No	No
Rhizobia MPN	No	**Yes**	No	Slight
Functional Tests				
N fixation	ND[†]	**Yes**	ND	ND
C metabolism (CLPP)	Slight	Slight	No	No
C catabolism (degradation)	No	No	No	No

N mineralisation	No	No	No	No
Potential nitrification	No	No	No	No

† ND not determined