Context dependent lotic macroinvertebrate responses in Michigan’s Upper and Lower Peninsulas to bioavailable sediment copper

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Abstract:

This research investigated context dependent responses of two macroinvertebrate communities to the same Cu treatments to see how community responses differed and changed with sediment aging and oxidation. Sites were located in Michigan's Upper (UP) and Lower Peninsulas (LP) that experience relatively low and high anthropogenic disturbance. We spiked clean sediments with Cu to establish five treatments (0-2100 mg/kg) and placed those sediments in two watersheds. Sediments were aged *in situ* for 12 weeks in the Pine (UP) and Little Molasses (LP) Rivers, then sampled at 1, 4, and 12 weeks for invertebrate colonization and geochemical composition. We found macroinvertebrate responses to Cu were context dependent and varied with site and season. We observed a 30% reduction in acid volatile sulfides (AVS) after 12 weeks due to oxidation. In turn Cu bound to FeO_x+MnO_x significantly increased after 12 weeks aging, which potentially decreased Cu bioavailability. This was supported by the significance of invertebrate metrics responding to Cu bound to Fe and Fe fractions in multiple regression analyses. We observed increased sediment oxidation after 12 weeks, which was likely the result of burrowing invertebrates at the Pine and sandy sedimentation at Little Molasses. Since we observed varied responses with only two sites, this suggests context dependency could play an important role in ecotoxicology and further research is needed addressing confounding issues of natural variation in ecotoxicology. We stress the need to incorporate FeO_x+MnO_x fractions in bioavailability models for oxic sediments in order to improve predictions of toxicity, as using sulfide and organic carbon solely to predict invertebrate responses can lead to the overestimation of toxicity when FeO_x+MnO_x fractions are present.

Keywords: copper partitioning, macroinvertebrate community ecology, ecotoxicology, redox environment

1. Introduction:

Freshwater sediments contaminated with metals can impair benthic communities causing degradation of aquatic ecosystems. Divalent metals (e.g. Cu, Zn, Pb) tend to adsorb to particulate matter and then settle out of solution and accumulate in sediments, where they are exposed to biogeochemically dynamic conditions (Lee *et al.* 2000; Eggleton & Thomas 2004; Kelderman & Osman 2007; Cantwell, Burgess & King 2008). Many divalent metals, such as copper, bind to a variety of chemical pools within sediments including sulfides, organic carbon (OC), and iron and manganese oxides (FeO_x+MnO_x; Allen, Fu & Deng 1993; Calmano, Hong & Forstner 1993; Kostka & Luther III 1994; Perin *et al.* 1997; Simpson, Apte & Batley 1998; Lee *et al.* 2000; Yu *et al.* 2001; Burton *et al.* 2005; Cantwell *et al.* 2008; Teuchies *et al.* 2010; Costello *et al.* 2011; De Jonge *et al.* 2012a). The bioavailable fraction of metal is the metal available for biological uptake and this fraction is often composed of metals dissolved in porewater (Eggleton & Thomas 2004; Burton 2010). Under equilibrium conditions it is predicted there will be a direct relationship between the metal concentrations in sediments, pore water, and benthic organisms (Burton 2010). This relationship has been defined procedurally by the formation of sulfide metal complexes. In molar excess, metals will displace Fe and Mn to form a stable precipitate with sulfides, rendering metals unavailable for biological uptake. Current methods estimate the bioavailable fractions in sediments by sulfide and metal concentrations, which are determined procedurally using a 1 N HCl acid extraction; the simultaneously extracted metal (SEM) in molar excess of acid volatile sulfides (AVS; $\Sigma SEM_{Me} - AVS$; Allen *et al.* 1993). Since metals bind readily to OC (Calmano *et al.* 1993; Perin *et al.* 1997; Cantwell *et al.* 2008), bioavailability models can be normalized by the fraction organic carbon in sediments ($(SEM_{Me} - AVS)/f_{OC}$) to improve estimations of bioavailable metals

(Burton 2010). These estimations are dependent on size of chemical pools (ligands available) and the partitioning coefficients, but also assume homogeneous, anoxic conditions, and a stable redox environment with sulfide binding dominating (Perin *et al.* 1997; Lee *et al.* 2000; Yu *et al.* 2001).

However, we know that the concentration of ligands (e.g. sulfide, OC, and FeO_x+MnO_x) are not stable and can vary spatially with redox potential (Perin *et al.* 1997; Eggleton & Thomas 2004) and temporally (van Griethuysen *et al.* 2006; Burton & Johnston 2010). In sediments an oxidized layer (oxic zones), defined by the penetration depth of O_2, lies just above the suboxic zone, in which oxidized species are present, but O_2 is not. Typically suboxic zones are defined by the presence of Fe oxides. These exist on top of reducing environments (anoxic zones), defined by the reduction of sulfate (Kristensen 2000). Oxic, suboxic, and anoxic zones create a dynamic redox environment affecting the partitioning of metals, such as Cu. Under anoxic conditions, sulfides form from microbially-mediated reduction of sulfate. In the presence of sulfides, CuS complexes form a highly insoluble precipitate (Allen *et al.* 1993; Simpson *et al.* 1998; DeJonge *et al.* 2012a). When anoxic sediments are exposed to O_2, sulfide oxidation occurs, which can release free Cu ions (Simpson *et al.* 1998; Lee *et al.* 2000; Teuchies *et al.* 2010; DeJonge *et al.* 2012a). In oxic and suboxic zones FeO_x+MnO_x are present (Kostka & Luther III 1994; Kristensen 2000), which can also bind metals thereby decreasing bioavailability in surface layers (Kelderman & Osman 2007; Costello *et al.* 2011; DeJonge *et al.* 2012a). The sizes of chemical pools are dynamic, shifting with changes in redox potential. Microbial metabolism accounts for the rapid declines in O_2 in surficial sediments, but is dependent on temperature, organic matter supply, current, and light (Kristensen 2000). Bioturbation (a process in which benthic organisms burrow, physically displacing anoxic sediment and increasing surface water transport) increases substrates for microbial degradation from secretions through mixing (Kristensen 2000; Meysman, Middelburg & Heip 2006). Bioturbation can significantly increase oxidation depths, but depend strongly on the invertebrate community composition and even season (e.g. presence of burrowers; Charbonneau & Hare 1998; Kristensen 2000).

Similarly, community responses to metals depend on context, in that the effects can vary along spatial, temporal, and environmental gradients (Clements, Hickey & Kidd 2012). Some taxa are known to be less tolerant of metal pollution than others (Burton & Johnston 2010). Ephemeroptera (mayfly), Plecoptera (stonefly), and Trichoptera (caddisfly) larvae (EPT) are sensitive to many stressors and these insects are commonly used as indicators of a host of environmental disturbances, including metal contamination, when they are present (Clements *et al.* 1989; Clements 1994; Clements & Kiffney 1995; Kiffney & Clements 1996; Costello *et al.* 2011). The amphipod *Hyalella azteca* is used for a wide range of toxicity testing and is sensitive to metals (Burton 1991; Burton *et al.* 2005; Costello *et al.* 2011). Another amphipod, *Gammarus spp.*, has also shown sensitivity to sediment Zn in previous studies (Costello *et al.* 2011). Other organisms (e.g. dipterans and oligochaetes) are readily abundant in the environment and are generally considered more tolerant of metal pollution (Clements *et al.* 1989; Clements & Kiffney 1995; DeJonge, Blust & Bervoets 2010; Costello *et al.* 2011). Communities with a high percentage of sensitive taxa might respond more readily to metal contamination than those largely composed of tolerant taxa; thus environmental context could inform predictions of toxicity (Clements *et al.* 1989, 2012; Clements & Kiffney 1995; DeJonge *et al.* 2012b).

Michigan's Upper and Lower Peninsulas offer a platform for comparing how different communities respond to Cu spiked sediments. These assemblages vary not only regionally, but also in disturbance regime. The Upper Peninsula site, Pine River (Marquette Co.), was located on privately owned land that is relatively undisturbed by human activities. The Lower Peninsula site, Little Molasses River (Gladwin Co.), is located in a state forest, which is well trafficked and prone to higher amounts of disturbances. The purpose of this study was firstly, to explore the responses of invertebrate communities that vary in ecological context to the same Cu treatments. Secondly, to monitor the invertebrate communities as sediments incubated *in situ* to see how responses changed. We further monitored physicochemical sediment characteristics in order to explore changes in Cu partitioning in sediments and explain why changes occurred with *in situ* aging. We expected to see context dependent responses to Cu and a greater magnitude of effect at the Pine, because of the low disturbance regime and high prevalence of EPT taxa. Secondly, we expect invertebrate responses to Cu to diminish with aging as Cu complexes with different pools of ligands and sediment oxidation increases, but for responses to continue to vary between sites.

2. Methods:

2.1. Sediment selection and Cu amendment:

Non-contaminated Raisin River depositional sediment was selected for Cu amendment because of its high AVS content (11.65 µmol g^{-1} dw). Surficial sediments were collected with shovels and stored under N_2 atmosphere at room temperature. Sediments were spiked with $CuCl_2 \cdot 2H_2O$ using the indirect-spiking method (Simpson, Angel & Jolley 2004; Hutchins *et al.* 2009; Brumbaugh *et al.* 2013). Briefly, a small volume of sediment was spiked with a high concentration of Cu, pH buffered, equilibrated for 2 weeks under N_2 atmosphere, and diluted to desired treatments (0, 380, 750, 1200, and 2100 mg Cu kg^{-1} dw). Both the superspike and dilutions were buffered with NaOH to maintain the pH within 0.5 units of initial sediment pH. Final sediment treatments were allowed to equilibrate under N_2 atmosphere for 14 days prior to deployment. All treatments were mixed twice a week (rolled for >1 hr.) to homogenize throughout the equilibration period.

Field contaminated sediments were collected in December 2011 from the Ocoee River floodplain in Polk Co., Tennessee. Historical mining activities from the mid 1800s until the late1980s caused metal contamination (predominantly Cu, secondarily Pb and Zn) of sediments (Carr & Zeller 2006). Sediments were chosen to represent a gradient of Cu contamination ranging from 170 – 1600 mg Cu kg^{-1} dw. Three of the sediment types were clay or clay/silt, while the lowest Cu concentration had a sandy texture. AVS concentrations also varied among the sediment types ranging from concentrations below detection limits to 10.39 µmol g^{-1} dw (Table 1).

2.2. Field deployment site description:

Two stream locations were selected for deployment of sediments for their variations in watershed and physicochemical characteristics, as well as differences in macroinvertebrate assemblages. The Pine River (P) in the Upper Peninsula (Marquette Co., MI) had a relatively undisturbed watershed, heterogeneous substrate, soft water (53 ± 4.16 mg L^{-1} $CaCO_3$; Briggs & Ficke 1977), and high abundance of sensitive EPT taxa (Yanoviak & McCafferty 1996). Little Molasses River (LM) in the Lower Peninsula (Gladwin Co., MI) had moderately hard to hard water (116 ± 23.4 mg L^{-1} $CaCO_3$; Briggs & Ficke 1977), homogeneous fine substrate (sand as opposed to cobble), and an abundance of sensitive amphipods (Costello *et al.* 2011; Honick 2013). Most importantly, macroinvertebrate community composition differed between sites. Background samples of depositional sediments from the Pine River had fewer taxa, higher diversity (Simpson's D), and greater evenness (Pielou's J). Little Molasses had more taxa than the Pine, but were dominated by fly larvae and Gammaridae leading to lower diversity and lower evenness (Pielou's J) when compared to the Pine (Table 2). Dissolved oxygen, temperature, conductivity, pH, and turbidity were measured hourly *in situ* throughout most of the 13 week experiment with datasondes (YSI 6920 V2). Surface water grab samples were collected 4 times—at deployment and each of the subsequent sampling days—to determine water hardness and alkalinity (Table 2).

2.3. Sediment deployment:

Sediments were deployed in July 2012 in the Little Molasses and Pine Rivers using *in situ* chambers (Burton *et al.* 2005; Costello *et al.* 2011; Honick 2013). Plastic chambers were made with plastic baskets (25.4 x 7.7 x 5.7 cm in dimension) lined with 1.5 mm mesh to prevent sediment loss (Fig. 1a). Chambers (n = 3 per treatment) were placed flush with the sediment surface and secured to the stream using steel frames and rebar (Fig. 1 a, b). Cu treatments were placed with lowest concentrations upstream of higher concentrations to minimize contamination due to sediment transport. Chambers were deployed in nylon mesh bags with openings of 5 mm (sufficient size for most macroinvertebrate colonization) to minimize sediment loss in times of high discharge (Fig. 1b).

2.4. Invertebrate and geochemical sampling:

Chambers were destructively collected to measure invertebrate colonization and geochemical composition after 1, 4, and 12 week aging periods in July, August, and October 2012 respectively. Each chamber was subdivided with two-thirds of the chamber reserved for invertebrate colonization and one third for geochemical sampling. The presumed biologically active layer of sediment (top 2 cm), was collected, sieved (45 µm), and preserved in 70% ethanol for identification (family level) and enumeration of macroinvertebrates (Hilsenhoff 1995; Merritt & Cummins 1996; Bouchard Jr. 2004). Invertebrate data was used to calculate community composition metrics including abundance, richness, EPT richness—defined as the number of EPT taxa (Lenat 1988; Merritt & Cummins 1996)—EPT abundance, relative EPT abundance, chironomid abundance, relative chironomid abundance, amphipod abundance, relative amphipod abundance, and Simpson’s diversity (1 – D; SI Table 1). The remaining sediment was submerged in a plastic container with site water and analyzed on site for sediment oxygen content at depth immediately after sampling using a microelectrode and motorized profiler set up within 100 feet of the sample site (Unisense OX 100). Dissolved oxygen (DO) concentrations were recorded every 500 µm until concentrations reached zero. The depth of the oxic layer was defined as the distance between the surface-water interface (i.e. the height just before DO concentrations began to decline) and the height at which DO concentrations first reached zero in the sediments (SI Fig. 1). The sediment reserved for geochemical analysis was divided into surface (top 2 cm) and deep (below 2 cm) fractions and stored frozen in plastic 50 mL centrifuge tubes for chemical analyses.

2.5. Chemical analyses:

All sediment samples were analyzed for AVS and SEM, total metals, organic carbon content (%OC), sediment water content (% dry weight), and FeO_x+MnO_x. AVS was extracted from wet sediment using a 1 N HCl acid volatilization process under anoxic conditions (Allen *et al.* 1993). Briefly, sulfides are volatilized, then trapped in NaOH, and measured colormetrically. SEM metals are those dissolved metals (0.45 µm filter) liberated during the acid volatilization of AVS. Total metals were extracted using concentrated acids (3:1 HNO_3: HCl) and microwave assisted digestion (U.S. EPA 2007). OC content was determined through measurement of organic matter content by loss on ignition (LOI) at 450°C and conversion to %OC via the Redfield ratio (0.36; Costello *et al.* 2011). Amorphous FeO_x+MnO_x were determined by incubating 0.1 – 0.5 g wet sediment in an ascorbate solution at room temperature on a shaker table for 24 hours and filtered (0.45 µm; Kostka & Luther III 1994). Cu-bound to FeO_x+MnO_x ($Cu_{ascorbate}$) were those metals liberated during the ascorbate extractions. All metal extractions were stored at room temperature and analyzed for Cu, Fe, and Mn on the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

2.6. Statistical analyses:

All statistical analyses were performed using R 2.14.1 (R Development Core Team 2011). Changes in sediment oxic layer depths were analyzed using a two-way analysis of variance (ANOVA) to determine how depths changed among treatments and with aging. Models were tested for normality using a Shapiro-Wilk test and if a significant model was observed ($\alpha = 0.05$) a TukeyHSD multiple comparisons post-hoc test (MCT) was performed to analyze the differences between factors. Changes to Cu partitioning during *in situ* aging were analyzed using a multi-way analysis of covariance (ANCOVA) to determine how relationships between pools of Cu and binding fractions (Cu_{total}, AVS, Cu_{SEM}, Fe_{SEM}, Mn_{SEM}, FeO_x+MnO_x, and $Cu_{ascorbate}$) changed though time with location as a block. Dependent variables were ln or square root transformed to meet the assumption of normality as determined by a Shapiro-Wilk test. A storm event caused deposition of sandy sediment on some replicates of Little Molasses Cu spiked chambers on week 4 (380, 750, 1200, and 2100 mg kg^{-1} surface sediments) and week 12 (750 and 1200 mg kg^{-1} surface and 1200 mg kg^{-1} deep sediments). This additional sand altered the sediment chemistry as evidenced by reductions in Fe_{total}, Mn_{total}, and Cu_{total} concentrations. For analyses of Cu partitioning during aging, those replicates were excluded from our statistical analysis to avoid the confounded factor of sediment burial (bear in mind patterns in week 4 surficial sediments reflect the Pine data only).

Multiple regression analysis with a forward stepping procedure was used to determine the geochemical or metal fractions that best predicted invertebrate metrics. Invertebrate colonization after week 1 was insufficient to characterize the communities (< 15 individuals for reference and spiked replicates) and was not included in multiple regression analyses. We did, however, include week 4 and 12 samples altered by sedimentation at Little Molasses (both response and predictor variables) in multiple regression analyses, because the disturbance did not appear to affect invertebrate colonization. All predictor variables were ln transformed prior to model stepping procedure except (Cu_{SEM}-AVS)/f_{OC} and Cu_{SEM}-AVS, for which negative values (which are predicted non-toxic; Burton 2010) were converted to zero and data were ln(x + 1) transformed. To account for differences in background community composition and potential variation in dose-response between sites, invertebrate metrics were analyzed with location as a potential factor or interaction term. When an interaction term was selected during the stepping procedure, main effects were only included in the final models if stepwise procedures selected them. Variables with the lowest significant p-value were added until there were no further significant variables (criteria $\alpha = 0.05$ for parameter inclusion). Given the high degree of correlation between deterministic variables, at each step all remaining variables were regressed against one another to account for multicollinearity. If the variables already in the model explained 50% or more of the variation in the new parameter, it was excluded from the model selection procedure as the variation inflation factor (VIF) ≥ 2 (Graham 2003). A single final best model was selected from the suite of stepwise models when all significant parameters were added to the model (Thompson 1978). Ocoee data were analyzed separately from Raisin sediments and qualitatively.

3. Results:

3.1. *Oxic sediment depth*

At the Pine River, the depth of the oxic layer differed significantly among sample weeks ($F_{[0.05, 2, 31]} = 42.583$, $p < 0.0001$), while at Little Molasses, once excluding samples altered by sedimentation, aging did not affect depth of the oxic layer. At the Pine River, depth of the oxic layer was similar between weeks 1 and 4 for all treatments. After 12 weeks *in situ* aging oxic layers were significantly deeper in lower treatments (0, 380, and 750 mg kg^{-1}; MCT, $p < 0.05$), while mean depths remained similar to earlier sampling periods in sediments treated with higher Cu concentrations (Fig. 2a). At Little Molasses River oxic layers were deeper in samples affected by sedimentation, while depths were not significantly different between other treatments and days (Fig. 2b).

3.2. *Cu partitioning in spiked sediments*

We observed statistically significant changes in redox sensitive sulfide species and simultaneously extracted Cu in surface sediments with sediment aging. As expected (Allen *et al.* 1993; Simpson *et al.* 1998), AVS was negatively correlated to Cu_{total} in surface ($t_{[0.05, 17]} = -10.053$, $p < 0.001$, Fig. 3a) and deep ($t_{[0.05, 22]} = -8.888$, $p < 0.001$; Fig. 3d) sediments. After 12 weeks *in situ* aging, we observed a 30% reduction in surficial AVS compared to previous weeks ($t_{[0.05, 17]} = -2.823$, $p = 0.01$) and a decrease in the magnitude of correlation between AVS and Cu_{total} ($t_{[0.05, 17}] = -2.392$, $p = 0.03$; Fig. 3a). Note that this is a conservative estimate of oxidation as oxic layers only include a fraction (10-50%) of total surface samples run for geochemical analysis. Cu_{SEM} was positively related to Cu_{total} in both surface ($t_{[0.05, 17]} = 10.017$, $p < 0.001$) and deep ($t_{[0.05, 22]} = 7.653$, $p < 0.001$) sediments. Week 1, Cu_{SEM} accounted for 55 ± 19% of Cu_{total}

and decreased to 43 ± 16% week 4 resulting in a significant decrease in the slope of the Cu_{SEM} and Cu_{total} relationship ($t_{[0.05, 17]} = -2.7$, $p = 0.02$; $t_{[0.05, 22]} = -2.291$, $p = 0.035$). Proportions returned to 49 ± 24%, similar to week 1 concentrations in week 12 (Fig. 3b,e).

Pools of FeO_x+MnO_x and associated Cu in surface sediments also changed as sediments aged *in situ*. FeO_x+MnO_x in deep sediments were not related to Cu_{total} and were homogenous between sample weeks and locations. Surface FeO_x+MnO_x concentrations were highest at week 1. We observed a 32% reduction in surface concentrations after 4 weeks ($t_{[0.05, 17]} = -2.9$, $p = 0.01$) and, compared to week 1, a 23% reduction after 12 weeks ($t_{[0.05, 17]} = -2.2$, $p = 0.04$). Concentrations of FeO_x+MnO_x in surface sediments at Little Molasses were 18% lower than concentrations at the Pine in surface sediments ($t_{[0.05, 17]} = -2.8$, $p = 0.01$). At Little Molasses concentrations of $Cu_{ascorbate}$ in surface sediments were significantly lower than at the Pine ($t_{[0.05, 17]} = -2.9$, $p = 0.009$). We observed a positive relationship between $Cu_{ascorbate}$ and Cu_{total} in both surface ($t_{[0.05, 17]} = 5.03$, $p = 0.0001$; Fig. 3c) and deep ($t_{[0.05, 22]} = 9.3$, $p < 0.000l$; Fig. 3f) sediments; the slope of that relationship significantly increased after week 12 in surficial sediments only ($t_{[0.05, 17]} = 2.8$, $p = 0.01$; Fig. 3c). The moles of FeO_x+MnO_x required for binding one mole of Cu also decreased with time. The mean molar ratio of FeO_x+MnO_x to $Cu_{ascorbate}$ for all treatments at both sites was greatest on week 1 (738.94± 316.2) and decreased weeks 4 (612.1 ± 322.4) and 12 (583.9 ± 340.8).

3.3. Macroinvertebrate colonization of Cu spiked chambers

Macroinvertebrates metrics strongly responded to sediment Cu after 4 weeks of aging but these effects diminished by week. After 4 weeks, 8 out of 10 macroinvertebrate metrics, including abundances, richness, and relative abundance, responded to at least one measure of sediment Cu pools (Table 3). No single Cu pool predicted all invertebrate responses as different metrics selected for $\mathrm{Cu_{SEM}}$, $\mathrm{Cu_{ascorbate}}$, and $(\mathrm{Cu_{SEM}}\text{-AVS})/f_{\mathrm{OC}}$ (Table 3). Total abundance, chironomid abundance, and amphipod abundance negatively responded to $\mathrm{Cu_{SEM}}$. At the Pine, EPT abundance negatively responded to $\mathrm{Cu_{ascorbate}}$ (Fig. 4e). Richness negatively responded to $(\mathrm{Cu_{SEM}}\text{-AVS})/f_{\mathrm{OC}}$ at Little Molasses (Fig. 5c). Relative amphipod abundance negatively responded to $\mathrm{Cu_{ascorbate}}$, while relative chironomid abundance did not respond to sediment Cu, but rather was negatively related to AVS (Fig. 6c, a). Gammaridae at Little Molasses drove the negative relationships between amphipod densities, relative amphipod abundance and Cu, because Gammaridae occur in higher densities than Hyalellidae. After 12 weeks *in situ* aging, we observed weakened relationships between the macroinvertebrate community and sediment Cu with only 3 out of 10 benthic metrics responding negatively to sediment Cu pools (Table 3). Abundance negatively responded to $\mathrm{Cu_{total}}$ (Fig. 4b) and diversity negatively responded to $\mathrm{Cu_{SEM}}$-AVS as a primary predictor and as a secondary predictor $\mathrm{Cu_{total}}$ at the Pine (Fig. 5b). Richness negatively responded to $(\mathrm{Cu_{SEM}}\text{-AVS})/f_{\mathrm{OC}}$ (Fig. 5d) and relative chironomid abundance positively responded to $\mathrm{Cu_{SEM}}$-AVS (Fig. 6b).

Responses of benthic macroinvertebrates to Cu were location dependent after 4 weeks of aging. Of the 10 metrics analyzed, 6 included location as a factor or interaction term with Cu (Table 3). In the Pine, abundance of EPT (Fig. 4e) and EPT richness (Fig. 5e) responded negatively to $\mathrm{Cu_{ascorbate}}$, while no significant relationship was observed at Little Molasses. Location alone explained greater diversity and relative EPT abundance at the Pine compared to Little Molasses (Fig. 5a; Fig. 6e), but neither responded to Cu. At Little Molasses relative amphipods, relative chironomids, and overall richness responded to Cu (Fig. 6c, a, 5c), while at the Pine no relationship was observed. We observed a decrease in EPT taxa at the Pine with increasing (Cu_{SEM}-AVS)/f_{OC}, while no relationship between EPT taxa and Cu was observed at Little Molasses (Fig. 5e). After 12 weeks of *in situ* aging, only 3 out of the 10 metrics included location as a factor or interaction term and none of the interactions were with measures of Cu. As a secondary variable diversity negatively responded to Cu_{total} at the Pine, while no relationship was observed Little Molasses (Table 3). .

Redox sensitive species, FeO_x+MnO_x, Fe_{SEM}, and AVS, fractions explained additional variation as primary and secondary parameters in chironomid, amphipod, and abundance models on weeks 4 and 12. After 4 weeks *in situ* aging AVS primarily and Fe_{SEM} secondarily explained variation in relative chironomids, while Fe_{SEM} (secondary to Cu_{SEM}) explained additional variation in chironomid abundance (Table 3). After 12 weeks FeO_x+MnO_x and Fe_{SEM} were primary predictors explaining variation in amphipod abundance and relative amphipods (which no longer responded to Cu; Fig. 4h, 6c). As a secondary predictor, FeO_x+MnO_x fractions explained variation in total abundance at both locations (Table 3).

3.4. *Macroinvertebrate colonization of field contaminated sediments*

Colonization of field contaminated sediments was driven by physicochemical characteristics and did not respond to Cu. Physicochemically, the field sediments include a wide range of textures and pH values. Ocoee1 was sandy in texture with a mean pH of 4.3 ± 1.2 and Ocoee 4 was clay with a mean pH of 5.4 ± 0.8. Ocoee 2 and 3 were also clay sediment textures with mean pH values of 4.2 ± 0.7 and 6.2 ± 0.8, respectively. Invertebrate colonization did not fit Cu bioavailability models, with high colonization of sediments at and above toxic thresholds (100 – 150 $\mu mol\ g_{oc}^{-1}$; Burton 2010) and low abundance below thresholds (Fig 7). Most of the invertebrates colonizing Ocoee chambers were epibenthic taxa (Gammaridae, Elmidae) or large predators (Gomphidae, Cambaridae).

4. Discussion:

Since the Pine and Little Molasses Rivers differed in disturbance regime, substrate heterogeneity, and macroinvertebrate community composition, we expected to see context dependent responses to Cu (Clements *et al.* 2012) and for the magnitude of response to be greater at the Pine. Background community composition did indeed drive invertebrate responses to Cu after 4 weeks *in situ* aging (sensitive EPT taxa at the Pine and Gammarus abundance at Little Molasses) and the magnitude of responses varied with sites (Table 3; Fig. 4, 5, 6). Rohr & Crumrine (2005) also observed that initial community composition affected freshwater community responses to pesticides in mesocosm experiments. Longitudinal variation is perhaps one of the most well documented examples of context dependent variation in stream ecology (Vannote *et al.* 1980), a concept which has been applied ecotoxicology. A previous mesocosm study found effects of metals were 12 – 85% greater at small headwater high altitude streams, suggesting responses to metals vary along longitudinal stream gradients (Kiffney & Clements 1996). However field collections suggest that natural changes in community composition that occur with elevation can interfere with determining if changes to community composition were directly effected by metals (Clements & Kiffney 1995). In this study variations in community composition alone (e.g. densities and relative sensitivity of EPT and amphipods) add a great deal of variability. Overlooking context dependent patterns in community composition could result in both over- or under-estimations of toxicity and choosing the correct metrics for the community is critical in assessing ecological effects. At Little Molasses, for instance, if only EPT metrics were analyzed, the assessor might over-estimate toxicological effects and would miss important shifts from relative Gammarus to chironomid dominance after 4 week exposures in response to Cu. Since invertebrate colonization is highly dependent on aquatic habitat and distribution of taxa tend to be conserved between similar substrate in lentic and lotic systems (Merritt & Cummins 1996), a perhaps more appropriate way to assess toxicity at local spatial scales would be to develop substrate specific indices for invertebrate responses in order to account for context dependent responses to metals.

We expected context dependent responses to persist over time; however invertebrate responses to Cu after 12 weeks aging were not location dependent (Table 3). This was surprising, but seasonal changes in community composition could account for these patterns. After 12 weeks, EPT and Gammarus densities decreased overall, suggesting community composition changed temporally. At Little Molasses we observed over a 50% reduction in reference densities between week 4 and 12 as a result of lower Gammarus densities. At the Pine, a reduction in EPT abundances was observed in both reference and spiked sediments after 12 weeks. Without accurate reference samples, one might conclude that reductions in EPT densities suggest effects of Cu persisted with aging. This could result in an over-estimate of toxicity, when seasonal variation could explain observed patterns. Temporal changes in community structures are important in assessing ecological risk; if this is neglected if could alter how we interpret field data. Broad community metrics (e.g. abundance, chironomid abundance, and relative chironomids) responded to Cu both days without location as a factor or interaction term, suggesting that at regional scales less resolute metrics may more accurately predict adverse responses to metals. Marchant, Barmuta & Chessman (1995) suggest that at large spatial scales family level taxonomic resolution may suffice, while at within-stream levels finer taxonomic resolution may be appropriate. A similar approach could be used in ecological risk assessment with community metrics to address some issues of ecological variation. Colonization of field contaminated sediments appear to respond to physicochemical characteristics rather than metal contamination, which highlights that factors other than contamination can lead to biological degradation (e.g. habitat destruction). Risk assessors should carefully consider these potential confounding factors so resources aren't misallocated for clean-up efforts when, for instance, habitat rehabilitation would suffice.

Oxidation of AVS after 12 weeks *in situ* aging occurred, while Cu binding to surficial FeO_x+MnO_x species increased in turn; potentially decreasing the fraction adversely affecting invertebrates (Fig. 3). Similar to previous studies we found Cu has a strong affinity for sulfides and OC, accounting for 43 – 55% of Cu partitioning in spiked sediments (Allen *et al.* 1993; Perin *et al.* 1997; Lee *et al.* 2000; Simpson *et al.* 2004; DeJonge *et al.* 2012a). Loss of AVS is similar to those observed in previous studies, where 8 – 12 hours after resusupension events or 18 – 54 days after incubation in oxic settings researchers observed 65 – 95% reductions in surficial AVS (Simpson *et al.* 1998; Lee *et al.* 2000; Teuchies *et al.* 2010; DeJonge *et al.* 2012a). However, we did not see an increase in adverse affects to invertebrates after 12 weeks, possibly due to increased concentrations of Cu scavenged by FeO_x+MnO_x. The invertebrate data supports that claim. Community metrics responded to Cu-bound FeO_x+MnO_x and Fe fractions even though this fraction accounted for 1 - 2% of Cu partitioning in sediments. Previous research suggests that higher proportions of Zn are found pooled in FeO_x+MnO_x fractions, while Cu tends to reside in OC and sulfide fractions (Kelderman & Osman 2007; DeJonge *et al.* 2012a). It is surprising that forward stepping procedures selected FeO_x+MnO_x and $Cu_{ascorbate}$ fractions to explain invertebrate colonization even though Cu has such a strong affinity for sulfides and OC. This suggests firstly, that redox sensitive Fe and Mn species are important fractions for predicting toxicity in oxic sediments and secondly, that perhaps Cu and OC or sulfide complexes are so stable that smaller fractions are driving community responses. Current lab based spiking methods use AVS to predict toxicity, yet two issues with these models arose in this study. Firstly, they fail to account for Cu bound to Fe fractions in surface sediments, which were important predictors of invertebrate colonization at the Pine and Little Molasses. Secondly, in this work multiple regression analysis more often selected Cu_{SEM} or $Cu_{ascorbate}$, suggesting that perhaps a weak acid and ascorbate extraction might be a cheaper and easier alternative for setting sediment quality guidelines in oxic sediments. These data suggest that short-term beaker tests on spiked anoxic sediments are not realistic comparisons to field conditions. In this work, Cu partitioning and oxic layer depths were significantly changing after 12 weeks in surficial sediments, suggesting that commonly used 14-day assays are insufficient to characterize long term community responses, especially under dynamic redox conditions.

Increases in oxic sediment depths at the Pine River were likely caused by invertebrate colonization, further altering the sediment redox environment. At the Pine, we observed increased oxic layer depth through time but the increase was not homogeneous across all treatments. Thus, water column oxygen concentrations in the stream (which were high and increased through time (data not shown)) were likely not driving the changes in redox stratification. Benthic organisms can increase oxic layer depths through bioturbation, by mixing anoxic, suboxic, and oxic layers and increasing sediment porosity (Kristensen 1984, 2000; Meysman *et al.* 2006). At the Pine, we found the burrowing mayfly Ephemeridae (*Hexagenia sp*.), and its abundance was greater in lower Cu sediments. Charbonneau and Hare (1998) found that *Hexagenia limbata* burrowed deeper in littoral sediments than all other burrowing taxa and estimate they alone could account for 98% of sediment displacement. Additional burrowing taxa (i.e., some chironomids, oligochaetes) were also observed in greater abundance on sediments with lower Cu concentrations. Furthermore, regardless of taxa, Charbonneau & Hare (1998) observed benthic organisms burrowed deeper in the autumn. It is likely that differences in the colonizing invertebrate community and seasonality may have contributed to changes in oxic layer depth through time at the Pine. There appears to be a positive feedback occurring at the Pine; deeper oxygen penetration causes oxidation of Fe species, further decreasing bioavailable Cu and potentially decreasing adverse affects on benthic taxa that further mix sediments. In contrast, the dominant taxa in lower treatments at Little Molasses were non-burrowing epibenthic organisms (Gammaridae), while benthic organisms (Chironomidae) were observed at lower densities than at the Pine. We observed increased oxic layer depths for only treatments affected by sand deposition, which is likely a result of differences in grain size. Increased grain size is directly related to greater porosity (Shepherd 1989), which leads to deeper oxygen penetration in sediments. This suggests that when epibenthic organisms dominate community composition, physical characteristics of the sediments might be determining sediment redox stratification. This is further evidence suggesting, firstly, short term toxicity tests are not sufficient to characterize Cu partitioning in sediments as oxic layer depths changed after 12 weeks aging and could be related to seasonal changes in invertebrate behaviors. Our data suggest that where burrowing organisms are present, determining the fraction of metals bound to redox sensitive fractions may be more important in assessing ecological risk. Toxicologists could use these patterns to their advantage. Sediments without benthic organisms might be at risk of more dramatic changes in partitioning as a result of disturbances, such as resusupension.

5. Conclusions:

This research investigated context dependent responses of macroinvertebrate communities to Cu and how community responses shifted with sediment aging and oxidation. We found macroinvertebrate responses were context dependent, varying with site and season. AVS oxidized after 12 weeks *in situ*, while Cu binding to surficial FeO_x+MnO_x increased. This potentially decreased the fraction adversely affecting invertebrates, which was supported by the prevalence of invertebrate metrics responding to Cu bound to Fe and Fe fractions in multiple regression analyses. Oxic layers deepened with aging, which might be the result of burrowing fauna at the Pine and increased grain size from sandy sedimentation at Little Molasses. We stress the need to incorporate FeO_x+MnO_x fractions in bioavailability models for oxic sediments in order to improve toxicity models. Current bioavailability models rely solely on sulfide and OC chemistry, but are missing important metal binding fractions, which could result in over-estimations of toxicity. Cu partitioning and oxic layer depths were significantly changing after 12 weeks in surface sediments, suggesting that commonly used short term tests (e.g. 14 day assays) are insufficient to characterize chronic responses, especially under dynamic redox conditions. This study was limited by a relatively narrow range of physicochemical characteristics (highlighted by the field contaminated sediments), as a single sediment type was used. Further research needs to be done with a wider range of sediment types to fully incorporate FeO_x+MnO_x into widely applicable models. Furthermore, we used only two sites to explore context dependent responses. Future research should examine context dependency with more sites to make broader suggestions for dealing with environmental context in ecotoxicology.

Table 1: Background sediment physicochemical properties. Reference sample total Cu was below the detection limits (b.d.) of the ICP-OES.

Sediment	AVS (µmol g^{-1} dw)	OC (%)	pH	Cu Trtmt	Tot. Cu	Tot. Pb	Tot. Zn	Tot. Fe	Tot. Mn
					(mg kg^{-1} dw)				
Raisin ref	10.3	3.2	7.1	ref	b.d.	---	---	---	---
Raisin 380	3.5	3.2	6.9	low	395	---	---	---	---
Raisin 750	1.2	3.2	6.9	med.	761	---	---	---	---
Raisin 1200	0.3	3.2	7.0	high	949	---	---	---	---
Raisin 2100	0.07	3.2	7.0	very high	1960	---	---	---	---
Ocoee 1	b.d.	0.5	3.2	ref	170	82	230	34	177
Ocoee 4	1.66	1.4	4.7	low	599	200	1098	55	655
Ocoee 2	0.02	1.6	3.4	medium	1199	439	1099	107	332
Ocoee 3	10.39	1.7	6.1	high	1600	210	1800	117	874

Table 2: Physical and biological community properties of the Pine (UP) and Little Molasses (LP) Rivers throughout sample aging. Data are presented as means and standard deviations. Temperature, dissolved oxygen, conductivity, pH, and turbidity were sampled hourly by datasondes throughout most of the 3 month aging period. Hardness and alkalinity were sampled 4 times at each deployment and sampling period. Biological communities were qualitatively assessed from depositional samples taken at deployment using a D-net.

	Pine R., UP	**Little Molasses R., LP**
Physical Properties		
latitude	N 46° 52’	N 43° 56’
longitude	W 87° 52’	W 84° 12’
temp (°C)	20.1 (3.8)[a]	16.7 (2.8)[c]
DO (% sat.)	97.8 (5.9)[b]	83.7 (7.2)[c]
conductivity (mS cm^{-1})	34.4 (19)[a]	92.1 (27.3)[d]
pH	7.7 (0.15)[a]	7.7 (0.23)[c]
turbidity (NTU)	0.82 (37.8)[a]	3.2 (10.4)[c]
hardness (mg $CaCO_3$ L^{-1})	53 (4.16)	116 (23.4)
alkalinity (mg $CaCO_3$ L^{-1})	54 (5.89)	101 (20.3)
Biological Properties		
richness (No.)	8	10
Shannon (H)	0.98	0.76
Simpson’s D (1-D)	0.72	0.33
Pielou (J)	1.52	0.76
Amphipods		
Hyalellidae (%)	30.2	0
Gammaridae (%)	0	9.8
Isopods (%)	27.9	0
Odonata		
Calopterygidae (%)	9.3	1.0
Aeshnidae (%)	13.9	0
Gomphidae (%)	7.0	0
Ephemeroptera		
Tricorythidae (%)	4.6	0
Baetidae (%)	0	<1
Heptageniidae (%)	0	<1
Diptera		
Chironomidae (%)	<1	81.1
Tabanidae (%)	0	3.8

[a] sampled hourly from 18 July to 4 October 2012
[b] sampled hourly from 24 July to 4 October 2012
[c] sampled hourly from 27 July to 18 September 2012
[d] sampled hourly from 11 August to 18 September 2012
[e] background samples taken at deployment of depositional areas

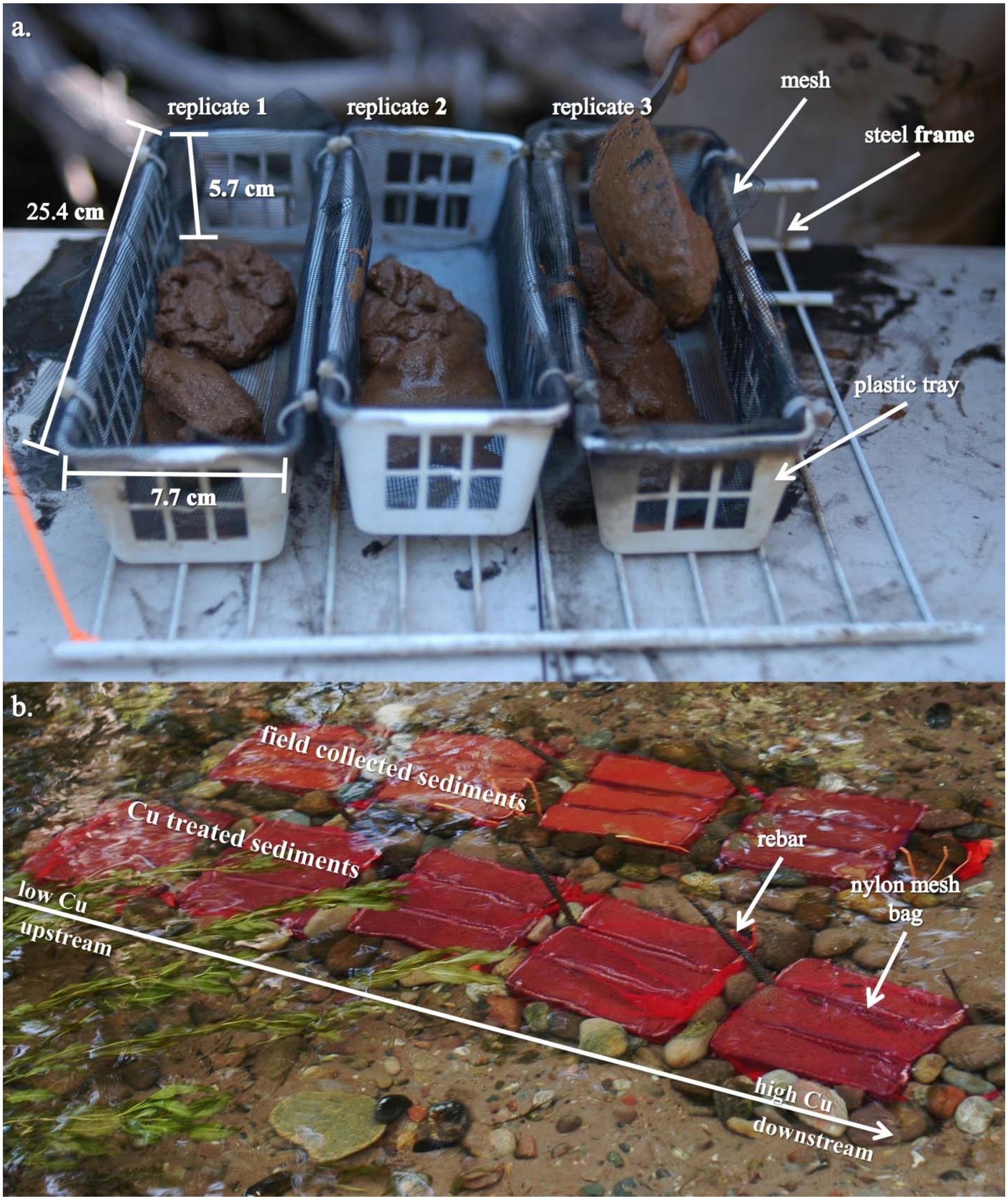


Figure 1: Diagram of sediment deployment set-up and chambers *in situ*. A) Chambers were deployed with 3 replicates for each treatment attached to a steel frame. B) Treatments were secured with rebar in the stream flush with the surface sediments in mesh nylon bags from low to high Cu concentration (upstream to downstream).

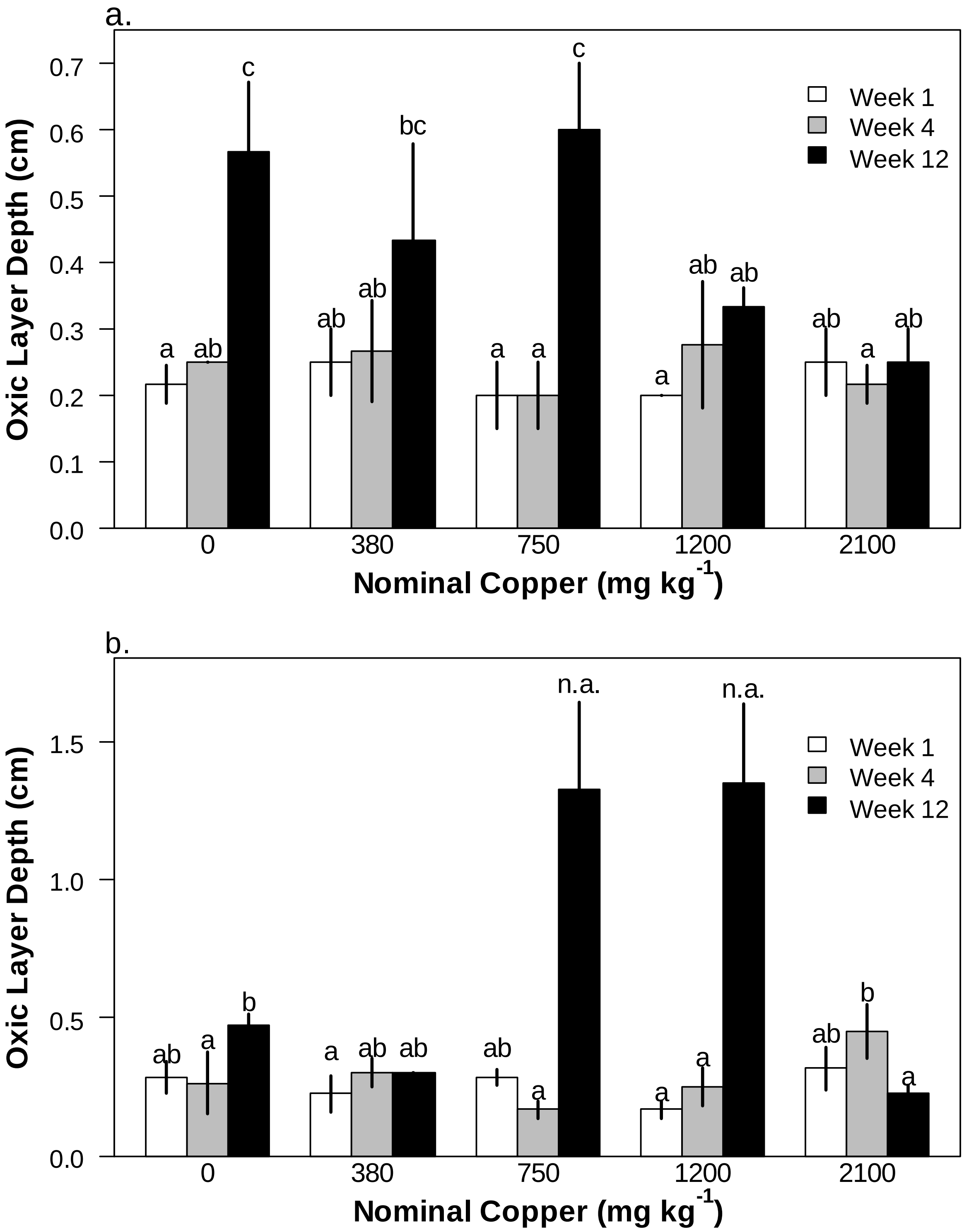


Figure 2: Oxic layer depth for copper spiked sediments aged in the Pine (a) and Little Molasses (b) Rivers. Samples were collected independently 1, 4, and 12 weeks after they were deployed in both locations designated by white, grey, and black representations respectively. Groups with the same letter designation are statistically homogeneous as determined by a MCT TukeyHSD test (α = 0.05). Little Molasses samples affected by sedimentation were removed from the statistical analyses, but are represented on the graph by n.a. designation. n = 46 (Pine), 40 (Little Molasses).

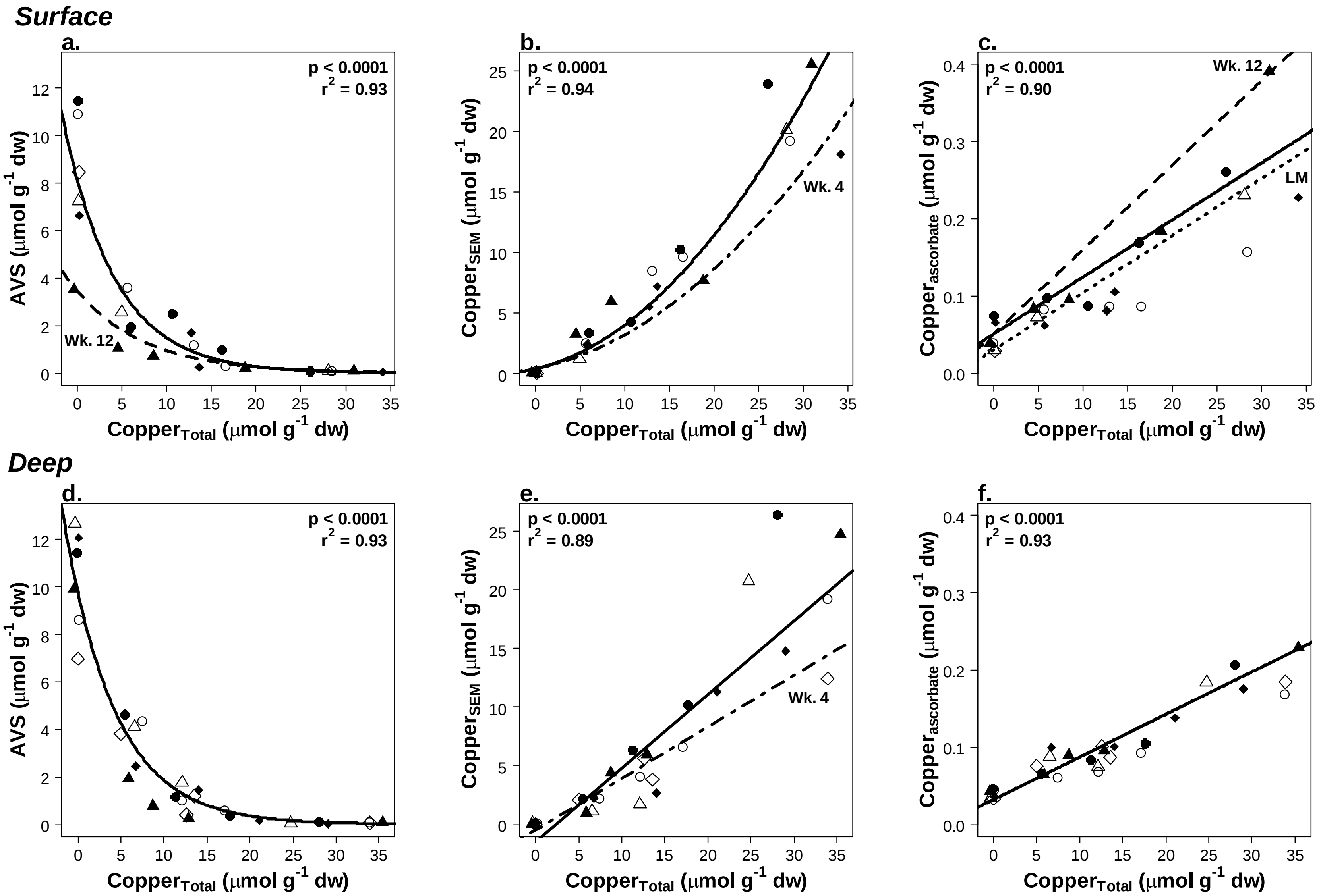
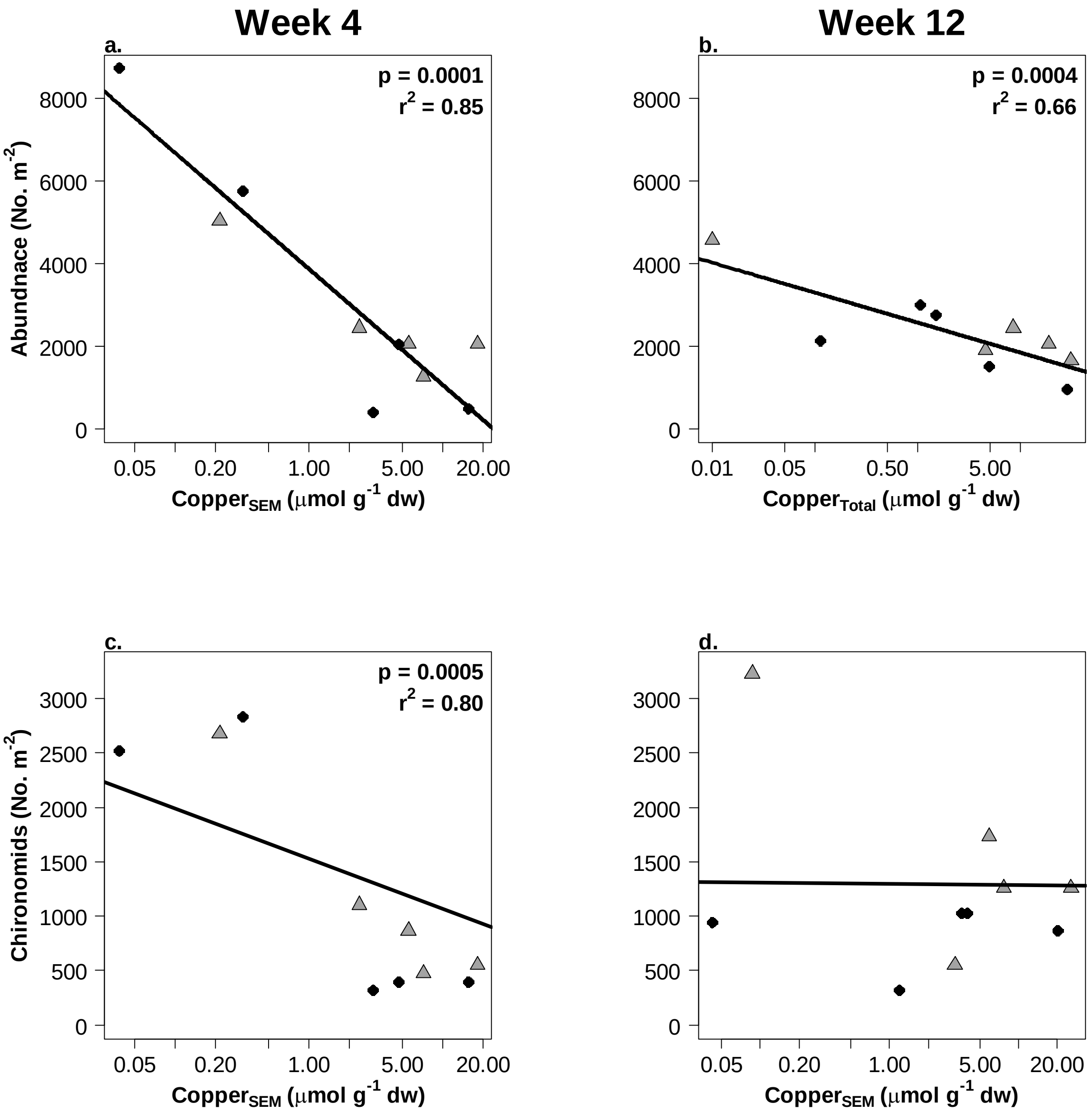


Figure 3: The relationship between total copper and AVS (a, d), Cu_{SEM} (b, e), and $Cu_{ascborbate}$ (c, f) fractions in surface and deep layers. Significant slopes as determined by ANCOVA are indicated as bold lines, while significant interactions and/or factors are indicated with dotted (factor LM), dot-dash (interaction week 4), and dashed (interaction or/and factor week 12) lines. Symbols represent the location and sampling period (Week 1 P [●], Week 1 LM[○], Week 4 P [♦], Week 4 LM [◊], Week 12 P [▲], Week 12 LM [△]). Models were run excluding samples altered by sedimentation at LM (surface n = 54; deep n = 59).

Table 3: Stepwise multiple regression (forward stepping) between benthic macroinvertebrate community metrics, copper, and sediment physicochemical factors. Columns indicate response variables and predictors are in 1° or 2° categories indicating the order in which factors were added to the model. Italicized benthic metrics identify terms with location interactions. Layer is indicated in parentheses (surface or deep) and directional effect are represented [-, +] for each metric including location in superscript (Little Molasses [LM], Pine [P]) where necessary. n = 10.

Response	1° Predictor	2° Predictor	r^2	p-value
Week 4				
abundance	Cu_{SEM} (surface) [-]		0.98	< 0.0001
chiron. ab.	Cu_{SEM} (surface) [-]	Fe_{SEM} (deep) [+]	0.92	0.0001
EPT ab.	*$Cu_{ascorbate}$ (deep) [-]^{P}*		0.86	0.001
amphipod ab.	Cu_{SEM} (deep) [-]		0.68	0.003
diversity (1-D)	Stream		0.70	0.002
taxa	*(Cu_{SEM}-AVS)/f_{OC}* (surface) *[-]^{LM}*	Stream	0.87	0.004
EPT taxa	*$Cu_{ascorbate}$ (deep) [-]^{P}*		0.69	0.02
Rel. chirono. ab.	*AVS (*surface*) [-]^{LM}*	*Fe_{SEM} (deep) [-]^{LM,P}*	0.95	0.001
Rel. amphipod ab	*$Cu_{ascorbate}$ (deep) [-]^{LM}*		0.72	0.01
Rel. EPT ab.	Stream		0.77	0.0008
Week 12				
abundance	Cu_{total} (surface) [-]	*FeO_x+MnO_x (deep) [-]^{LM,P}*	0.64	0.03
chiron. ab.				
EPT ab.				
amphipod ab.	*FeO_x+MnO_x (deep) [-]^{LM,P}*		0.83	0.002
diversity (1-D)	(Cu_{SEM}-AVS) (deep) [-]	*Cu_{total} (deep) [-]^{P}*	0.64	0.006
taxa	(Cu_{SEM}-AVS)/f_{OC} (deep) [-]		0.45	0.03
EPT taxa	Stream		0.41	0.05
Rel. chirono. ab.	(Cu_{SEM}-AVS) (deep) [+]		0.54	0.02
Rel. amphipod ab	*Fe_{SEM} (*surface*)[-]^{LM,P}*		0.80	0.004
Rel. EPT ab.				


Week 4
Week 12
a.
p = 0.0001
r2 = 0.85
Abundnace (No. m-2)
8000
6000
4000
2000
0
0.05
0.20
1.00
5.00
20.00
CopperSEM (µmol g-1 dw)
b.
p = 0.0004
r2 = 0.66
8000
6000
4000
2000
0
0.01
0.05
0.50
5.00
CopperTotal (µmol g-1 dw)
c.
p = 0.0005
r2 = 0.80
Chironomids (No. m-2)
3000
2500
2000
1500
1000
500
0
0.05
0.20
1.00
5.00
20.00
CopperSEM (µmol g-1 dw)
d.
3000
2500
2000
1500
1000
500
0
0.05
0.20
1.00
5.00
20.00
CopperSEM (µmol g-1 dw)

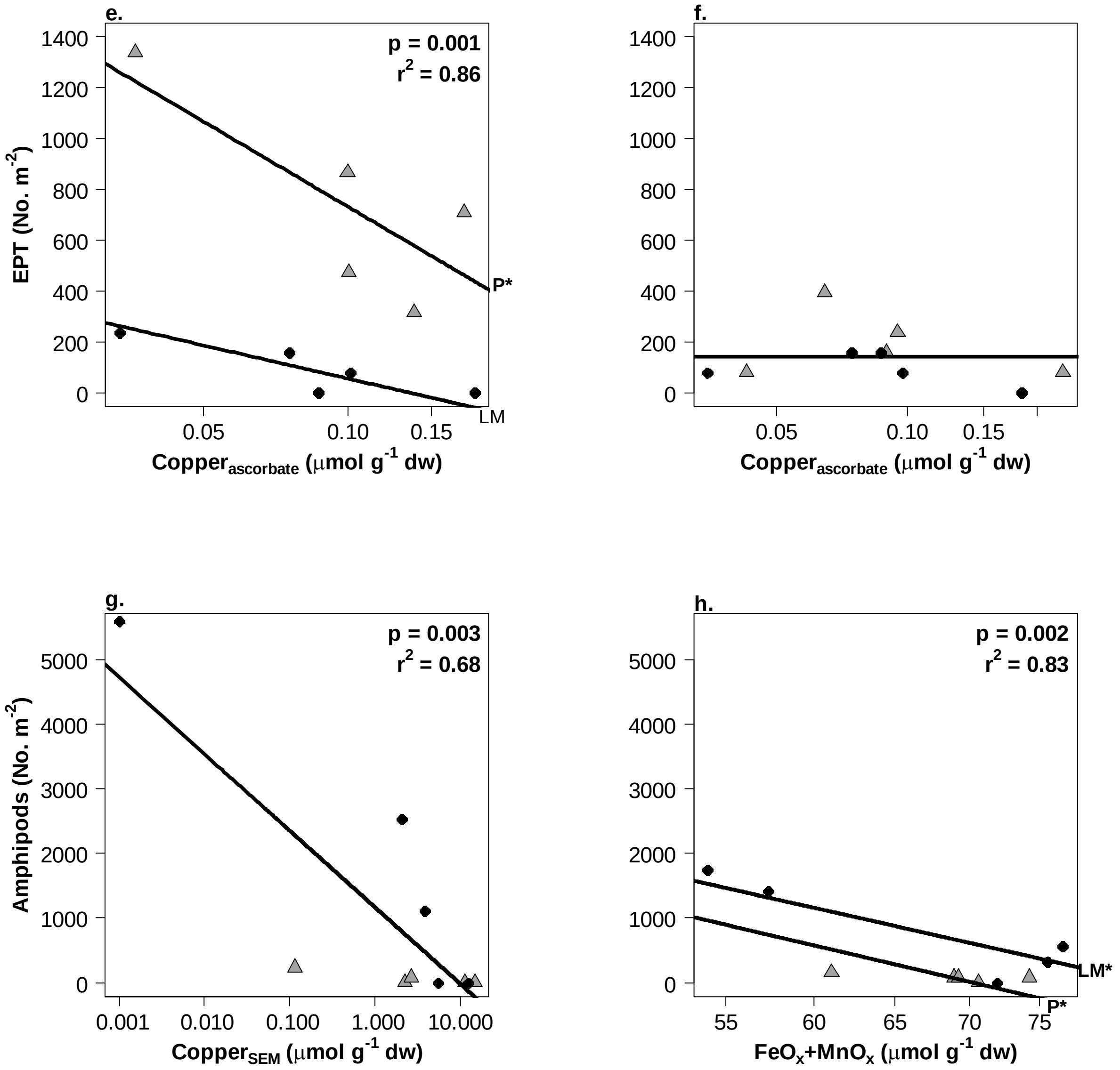
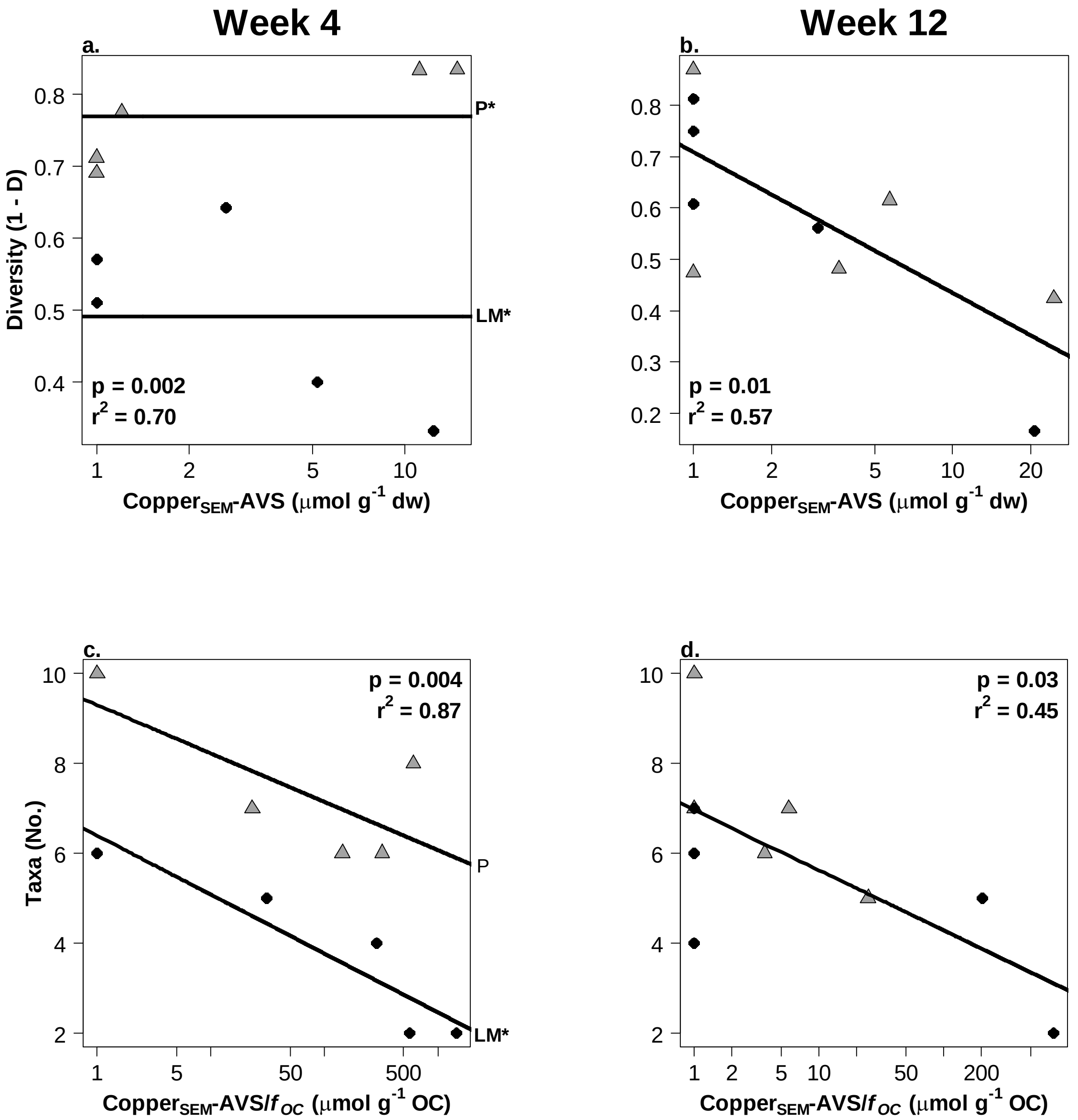


Figure 4: Response of benthic macroinvertebrate abundances to Cu and FeO_x+MnO_x after 4 and 12 weeks *in situ* aging. Abundance (a., b.), chironomids (c., d.), EPT (e., f.), and amphipods (g., h) responded to Cu_{total}, Cu_{SEM}, $Cu_{ascorbate}$, or FeO_x+MnO_x indicated on the x-axis (note the ln scale). Bold lines represent slopes predicted from forward stepping models. For models incorporating interactions Pine (P) and Little Molasses (LM) responses are included with asterisks to indicate significant slopes. n = 10.


Week 4
Week 12
a.
b.
c.
d.
Diversity (1 - D)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
P*
LM*
p = 0.002
r² = 0.70
p = 0.01
r² = 0.57
1
2
5
10
20
Copper_SEM-AVS (µmol g⁻¹ dw)
Taxa (No.)
10
8
6
4
2
P
LM*
p = 0.004
r² = 0.87
p = 0.03
r² = 0.45
1
5
50
500
1
2
5
10
50
200
Copper_SEM-AVS/f_OC (µmol g⁻¹ OC)

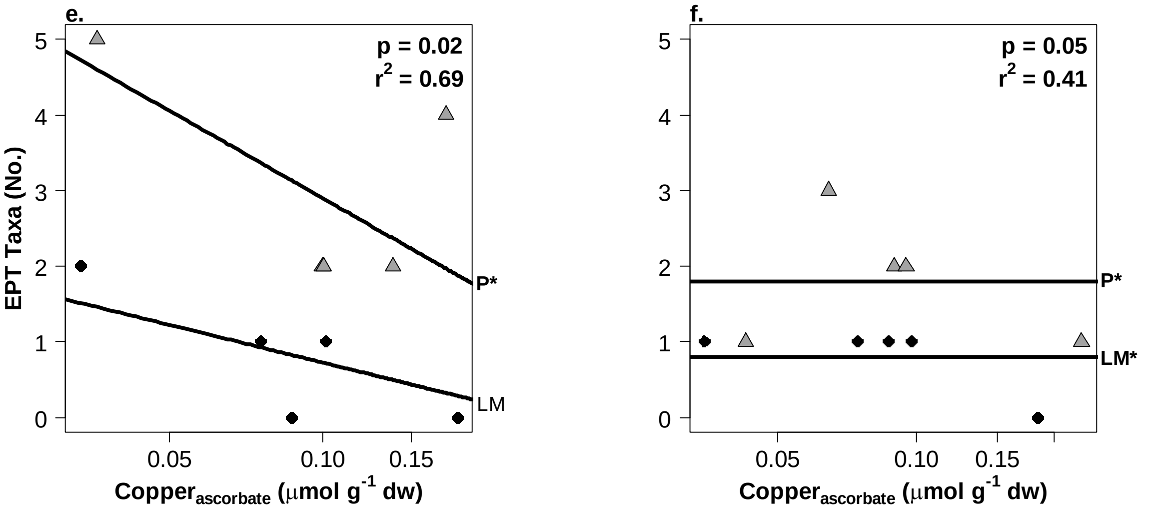
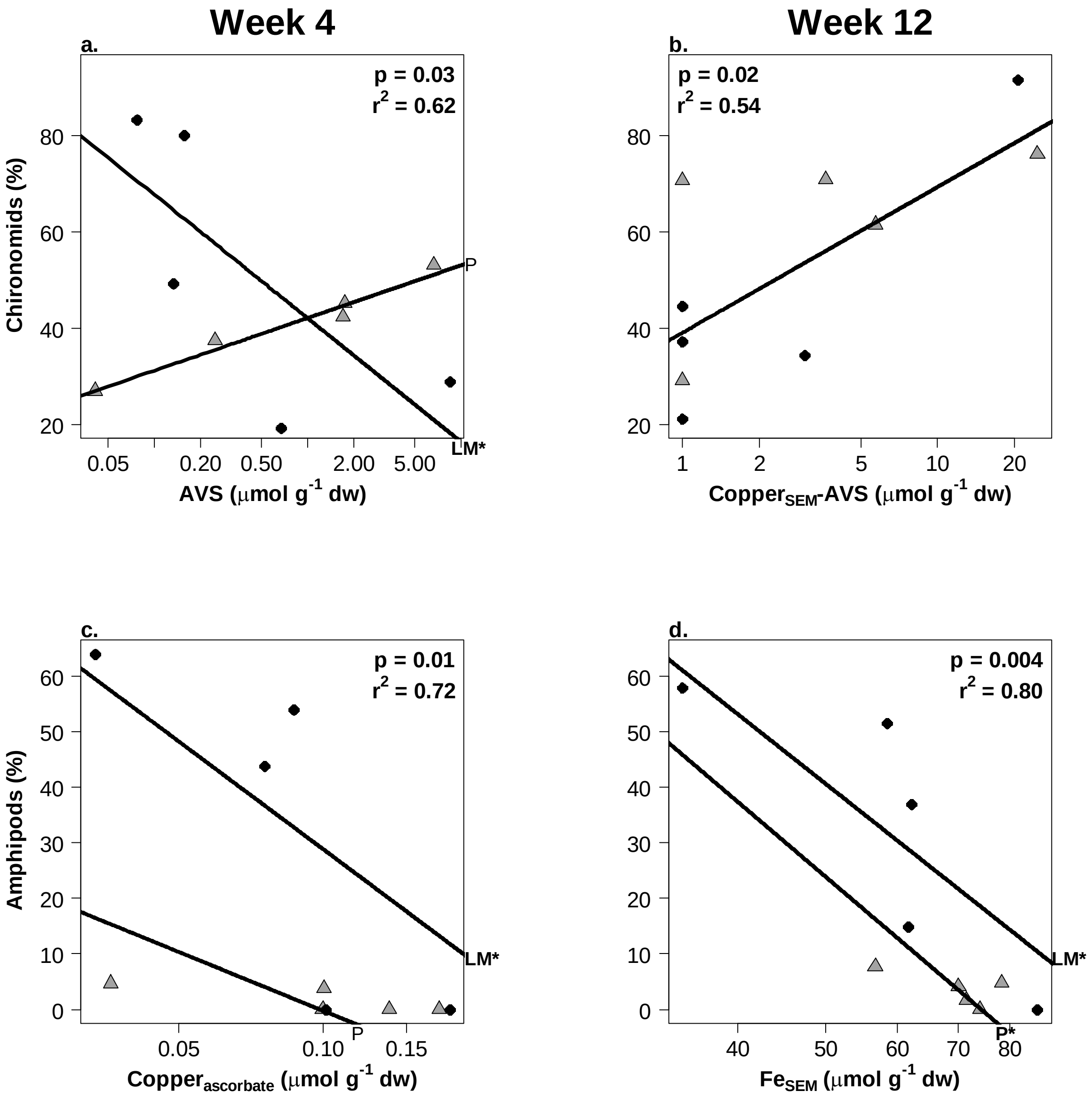


Figure 5: Response of benthic macroinvertebrate diversity and richness to Cu after 4 and 12 weeks *in situ* aging. Diversity (a., b.), taxa (c., d.), and EPT taxa (e., f) responded to Cu$_{SEM}$-AVS, Cu$_{SEM}$-AVS/f_{OC}, or Cu$_{ascorbate}$ indicated on the x-axis (note the ln scale). Bold lines represent slopes predicted from forward stepping models. For models incorporating interactions Pine (P) and Little Molasses (LM) responses are included with asterisks to indicate significant slopes. In models containing location solely as factor, significant intercepts are indicated by asterisks next to the location designation. n = 10.


Week 4
Week 12
a.
p = 0.03
r² = 0.62
Chironomids (%)
80
60
40
20
P
LM*
0.05
0.20
0.50
2.00
5.00
AVS (μmol g⁻¹ dw)
b.
p = 0.02
r² = 0.54
1
2
5
10
20
Copper_SEM-AVS (μmol g⁻¹ dw)
c.
p = 0.01
r² = 0.72
Amphipods (%)
60
50
40
30
20
10
0
LM*
P
0.05
0.10
0.15
Copper_ascorbate (μmol g⁻¹ dw)
d.
p = 0.004
r² = 0.80
LM*
P*
40
50
60
70
80
Fe_SEM (μmol g⁻¹ dw)

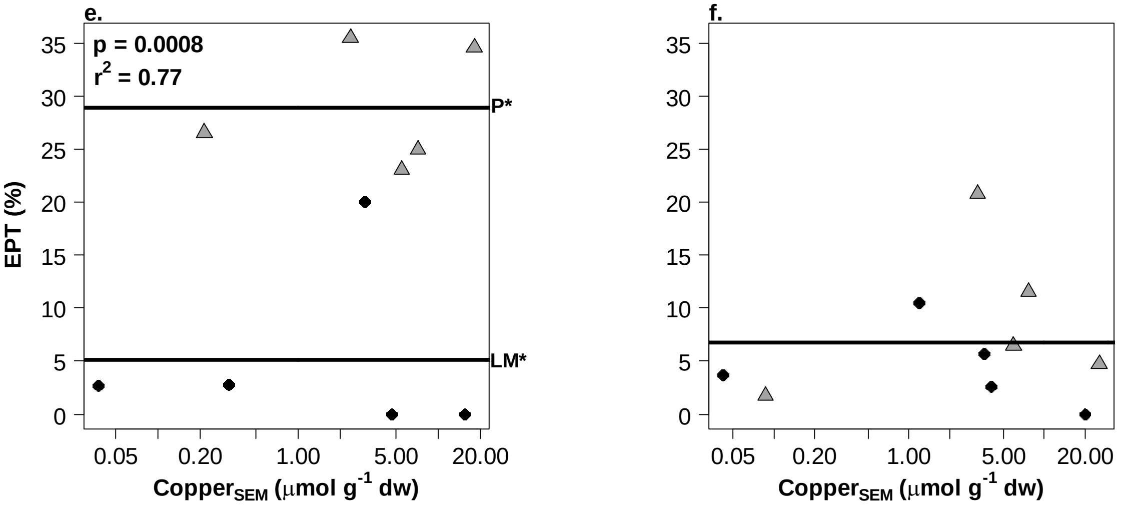


Figure 6: Response of benthic macroinvertebrate percent community composition to Cu, AVS, and Fe after 4 and 12 weeks *in situ* aging. Relative chironomid abundance (a., b.), relative amphipod abundance (c., d.), and relative EPT abundance (e., f) responded to Cu_{SEM}-AVS, Cu_{SEM}, $Cu_{ascorbate}$, AVS, or Fe_{SEM} indicated on the x-axis (note the ln scale. Bold lines represent slopes predicted from forward stepping models. For models incorporating interactions Pine (P) and Little Molasses (LM) responses are included with asterisks to indicate significant slopes. In models containing location solely as factor, significant intercepts are indicated by asterisks next to the location designation. n = 10.

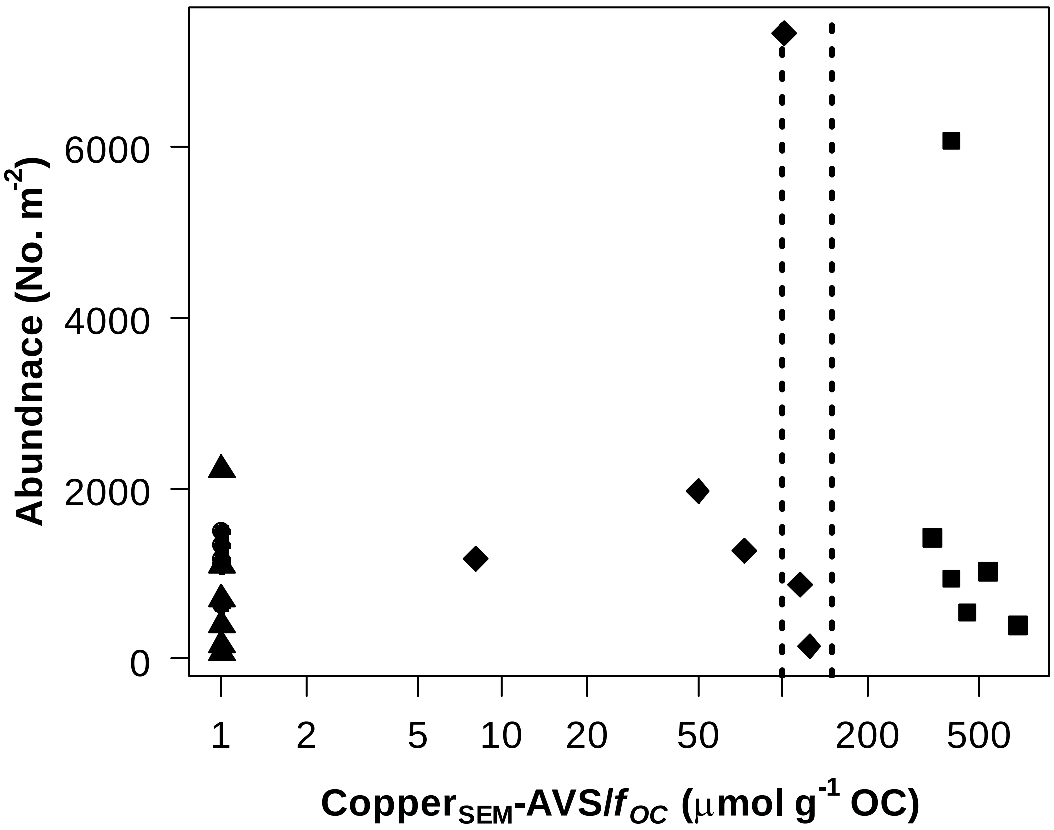


Figure 7: Invertebrate colonization of field contaminated sediments in relation to bioavailable Cu ((Cu_{SEM} – AVS)/f_{OC}). Different symbols represent the each sediment (Ocoee 1 [♦], Ocoee 4 [▲], Ocoee 2 [■], Ocoee 3 [●]). Note the ln scale on the x-axis. Dotted lines represent the toxic threshold for chronic responses (100 – 150 µmol/g_{oc}).

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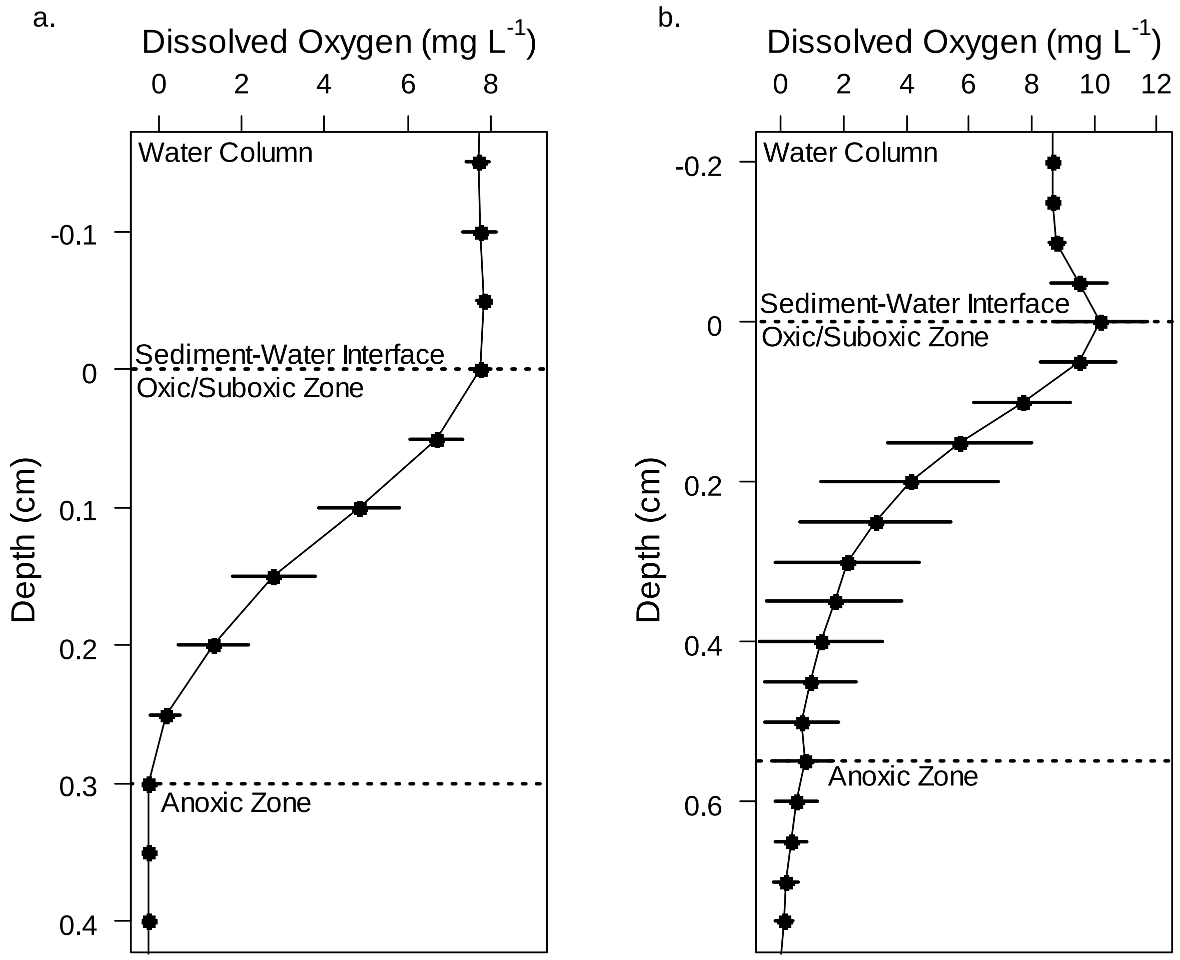
Supporting Information:

SI Table 1: Description of benthic macroinvertebrate indices.

Benthic Metric	Metric Description	citation
abundance (No. m^{-2})	The total number of individuals divided by the area of the chambers sampled (0.0127 m^2).	Klemm *et al.* 1990; Cummins & Merritt. 1996; Plafkin *et al.* 1989
chironomid abundance (No. m^{-2})	The total number of Chironomidae individuals divided by the area of the chambers sampled (0.0127 m^2).	Klemm *et al.* 1990; Cummins & Merritt. 1996; Plafkin *et al.* 1989
EPT abundance (No. m^{-2})	The total number of Ephemeroptera, Plecoptera, and Trichoptera individuals divided by the area of the chambers sampled (0.0127 m^2).	Klemm *et al.* 1990; Cummins & Merritt. 1996; Plafkin *et al.* 1989
amphipod abundance (No. m^{-2})	The total number of Amphipoda individuals divided by the area of the chambers sampled (0.0127 m^2).	Klemm *et al.* 1990; Cummins & Merritt. 1996; Plafkin *et al.* 1989
diversity (1-D)	Simpson's D (presented as 1 – D): $D=\sum[n(n-1)])/(N(N-1))$ where n is the number of individuals in each family and N is the total number of individuals in the sample. Simpson index is only applicable if $2 \leq N$.	Simpson 1949; Magurran 2004; Klemm *et al.* 1990
taxa (No.)	Number of separate taxa key to the family level when possible.	Lenat 1988; Klemm *et al.* 1990
EPT taxa (No.)	Number of separate EPT taxa key to the family level when possible.	Lenat 1988; Cummins & Merritt. 1996
relative chironomids (%)	Percent individuals in the total sample that belong to the Chironomidae family	Klemm *et al.* 1990
relative amphipod (%)	Percent individuals in the total sample that belong to the Amphipoda order	Klemm *et al.* 1990
relative EPT (%)	Percent individuals in the total sample that belong to the orders Ephemeroptera, Plecoptera, and Trichoptera.	Klemm *et al.* 1990



SI Figure 1: Depth profiles of sediment dissolved oxygen (DO) concentrations from week 4 Raisin reference (a) and Ocoee 1 (b). Zero represents the sediment-water interface, determined as the point at which DO decreased (represented by the top dotted line at 0). The increase DO in panel b is due to primary producers growing on the surface sediments. Points are represented as the mean and standard deviation of all replicates at that depth (a. n = 3; b. n = 5). The depth of the oxic layer was calculated for each replicate separately, from which a mean for each treatment was determined (represented by the dotted lines at 0.3 and 0.54 respectively).

Appendix:

Table A1: Summary of 2-way ANOVA statistics of Raisin mean oxic layer depths. Samples affected by sedimentation at Little Molasses were not included in the analyses (Pine: n = 46, LM: n = 40).

	df	Sum Sq.	Mean Sq.	F value	p-value	
Pine						
DAY	2	0.4190	0.20948	42.583	**< 0.0001**	***
CU	4	0.0697	0.01743	3.543	**0.017**	*
DAY:CU	8	0.2188	0.02735	5.560	**0.0002**	***
Residuals	31	0.1525	0.00492			
Little Molasses						
DAY	2	0.01785	0.008925	2.264	0.12	
CU	4	0.06658	0.016644	4.221	**0.009**	**
DAY:CU	6	0.18886	0.031477	7.983	**< 0.0001**	***
Residuals	27	0.10646	0.003943			

Table A2: Summary of Tukey post-hoc multiple comparison test from ANOVA statistics of Raisin mean oxic layer depths. Samples affected by sedimentation at Little Molasses were not included in the analyses and represented by NA (Pine: n = 46, LM: n = 40).

Comparison	**Pine p adj**	**Little Molasses p adj**
Day28:Ref - Day6:Ref	0.9999988	1
Day88:Ref - Day6:Ref	**0.0000775**	0.1115762
Day6:380 - Day6:Ref	0.9999988	0.9943185
Day28:380 - Day6:Ref	0.9998387	1
Day88:380 - Day6:Ref	**0.0387505**	1
Day6:750 - Day6:Ref	1	1
Day28:750 - Day6:Ref	1	0.6143701
Day88:750 - Day6:Ref	**0.0000156**	NA
Day6:1200 - Day6:Ref	1	0.6143701
Day28:1200 - Day6:Ref	0.9982195	0.9999987
Day88:1200 - Day6:Ref	0.762505	NA
Day6:2100 - Day6:Ref	0.9999988	0.9999947
Day28:2100 - Day6:Ref	1	0.1344645
Day88:2100 - Day6:Ref	0.9999988	0.9943185
Day88:Ref - Day28:Ref	**0.0003885**	**0.0325249**
Day6:380 - Day28:Ref	1	0.9998779
Day28:380 - Day28:Ref	1	0.9999503
Day88:380 - Day28:Ref	0.141076	0.9999503
Day6:750 - Day28:Ref	0.9998387	1

Day28:750 - Day28:Ref	0.9998387	0.7827535
Day88:750 - Day28:Ref	**0.0000775**	NA
Day6:1200 - Day28:Ref	0.9998387	0.7827535
Day28:1200 - Day28:Ref	0.9999999	1
Day88:1200 - Day28:Ref	0.9740397	NA
Day6:2100 - Day28:Ref	1	0.9972226
Day28:2100 - Day28:Ref	0.9999988	**0.03238**
Day88:2100 - Day28:Ref	1	0.9998779
Day6:380 - Day88:Ref	**0.0003885**	**0.0061658**
Day28:380 - Day88:Ref	**0.0008663**	0.1961057
Day88:380 - Day88:Ref	0.5791849	0.1961057
Day6:750 - Day88:Ref	**0.0000347**	0.1115762
Day28:750 - Day88:Ref	**0.0000347**	0.0008511
Day88:750 - Day88:Ref	0.9999988	NA
Day6:1200 - Day88:Ref	**0.0000347**	**0.0008511**
Day28:1200 - Day88:Ref	**0.0004911**	0.0673723
Day88:1200 - Day88:Ref	**0.0190069**	NA
Day6:2100 - Day88:Ref	**0.0003885**	0.3226029
Day28:2100 - Day88:Ref	**0.0000775**	1
Day88:2100 - Day88:Ref	**0.0003885**	**0.0061658**
Day28:380 - Day6:380	1	0.952804
Day88:380 - Day6:380	0.141076	0.952804
Day6:750 - Day6:380	0.9998387	0.9943185
Day28:750 - Day6:380	0.9998387	0.9943185
Day88:750 - Day6:380	**0.0000775**	NA
Day6:1200 - Day6:380	0.9998387	0.9943185
Day28:1200 - Day6:380	0.9999999	0.9999999
Day88:1200 - Day6:380	0.9740397	NA
Day6:2100 - Day6:380	1	0.8280993
Day28:2100 - Day6:380	0.9999988	**0.0048729**
Day88:2100 - Day6:380	1	1
Day88:380 - Day28:380	0.2458419	1
Day6:750 - Day28:380	0.9965195	1
Day28:750 - Day28:380	0.9965195	0.4111306
Day88:750 - Day28:380	**0.0001736**	NA
Day6:1200 - Day28:380	0.9965195	0.4111306
Day28:1200 - Day28:380	1	0.9998236
Day88:1200 - Day28:380	0.9965195	NA
Day6:2100 - Day28:380	1	1
Day28:2100 - Day28:380	0.9998387	0.2460511
Day88:2100 - Day28:380	1	0.952804
Day6:750 - Day88:380	**0.0190069**	1
Day28:750 - Day88:380	**0.0190069**	0.4111306

Day88:750 - Day88:380	0.2458419	NA
Day6:1200 - Day88:380	**0.0190069**	0.4111306
Day28:1200 - Day88:380	0.2265443	0.9998236
Day88:1200 - Day88:380	0.9015432	NA
Day6:2100 - Day88:380	0.141076	1
Day28:2100 - Day88:380	**0.0387505**	0.2460511
Day88:2100 - Day88:380	0.141076	0.952804
Day28:750 - Day6:750	1	0.6143701
Day88:750 - Day6:750	**0.000007**	NA
Day6:1200 - Day6:750	1	0.6143701
Day28:1200 - Day6:750	0.9811439	0.9999987
Day88:1200 - Day6:750	0.5791849	NA
Day6:2100 - Day6:750	0.9998387	0.9999947
Day28:2100 - Day6:750	1	0.1344645
Day88:2100 - Day6:750	0.9998387	0.9943185
Day88:750 - Day28:750	**0.000007**	NA
Day6:1200 - Day28:750	1	1
Day28:1200 - Day28:750	0.9811439	0.9730778
Day88:1200 - Day28:750	0.5791849	NA
Day6:2100 - Day28:750	0.9998387	0.2460511
Day28:2100 - Day28:750	1	**0.0005836**
Day88:2100 - Day28:750	0.9998387	0.9943185
Day6:1200 - Day88:750	**0.000007**	NA
Day28:1200 - Day88:750	**0.0000877**	NA
Day88:1200 - Day88:750	**0.0041989**	NA
Day6:2100 - Day88:750	**0.0000775**	NA
Day28:2100 - Day88:750	**0.0000156**	NA
Day88:2100 - Day88:750	**0.0000775**	NA
Day28:1200 - Day6:1200	0.9811439	0.9730778
Day88:1200 - Day6:1200	0.5791849	NA
Day6:2100 - Day6:1200	0.9998387	0.2460511
Day28:2100 - Day6:1200	1	**0.0005836**
Day88:2100 - Day6:1200	0.9998387	0.9943185
Day88:1200 - Day28:1200	0.9982195	NA
Day6:2100 - Day28:1200	0.9999999	0.9962976
Day28:2100 - Day28:1200	0.9982195	0.0824583
Day88:2100 - Day28:1200	0.9999999	0.9999999
Day6:2100 - Day88:1200	0.9740397	NA
Day28:2100 - Day88:1200	0.762505	NA
Day88:2100 - Day88:1200	0.9740397	NA
Day28:2100 - Day6:2100	0.9999988	0.4111306
Day88:2100 - Day6:2100	1	0.8280993
Day88:2100 - Day28:2100	0.9999988	**0.0048729**

Table A3: Description of ANCOVA results modeling Cu fractions and sediment physicochemical fractions as a function of total Cu and Time with Location as a block. Transformations are noted in model equations and were determined by a Shapiro-Wilk test for normality.

Model					**df**	**F-Value**	**R^2**	**p-value**
Surface								
ln(AVS) ~ Cu$_{total}$ * Time + Location					6 and 17	37.24	0.93	< 0.0001
	Coeffic.	t- value	p - value					
Alpha	**2.3**	**7.281**	**$1.28e^{-6}$**	***				
Cu$_{TOT}$	**-0.19**	**-10.053**	**$1.43e^{-8}$**	***				
Day 28	-0.57	-1.326	0.2025					
Day 88	**-1.1**	**-2.823**	**0.0117**	*				
LM	0.26	1.050	0.3083					
Cu$_{TOT}$:Day 28	0.04	1.468	0.1604					
Cu$_{TOT}$:Day 88	**0.06**	**2.392**	**0.0286**	*				
sqrt(Cu$_{SEM}$) ~ Cu$_{total}$ * Time + Location					6 and 17	43.78	0.94	< 0.0001
	Coeffic.	t- value	p - value					
Alpha	-1.14	-0.88	0.391					
Cu$_{TOT}$	**0.78**	**10.017**	**$1.51e^{-8}$**	***				
Day 28	1.01	0.571	0.576					
Day 88	0.41	0.243	0.811					
LM	-0.64	-0.621	0.543					
Cu$_{TOT}$:Day 28	**-0.26**	**-2.291**	**0.035**	*				
Cu$_{TOT}$:Day 88	-0.03	-0.321	0.752					
Cu$_{ascorbate}$~ Cu$_{total}$ * Time + Location					6 and 17	26.84	0.90	< 0.0001
	Coeffic.	t- value	p - value					
Alpha	**0.07**	**3.891**	**0.0011**	**				
Cu$_{TOT}$	**0.005**	**5.028**	**0.0001**	***				
Day 28	-0.19	-0.807	0.43					
Day 88	-0.023	-1.027	0.31					
LM	**-0.04**	**-2.940**	**0.009**	**				
Cu$_{TOT}$:Day 28	-0.0006	-0.376	0.711					
Cu$_{TOT}$:Day 88	**0.004**	**2.781**	**0.012**	*				
Fe$_{SEM}$ ~ Cu$_{total}$ * Time + Location					6 and 17	1.338	032	0.29
Mn$_{SEM}$ ~ Cu$_{total}$ * Time + Location					6 and 17	1.998	0.41	0.122
FeO$_x$+MnO$_x$ ~ Cu$_{total}$ * Time + Location					6 and 17	3.47	0.55	0.02
	Coeffic.	t- value	p - value					
alpha	**98.884**	**15.35**	**<0.0001**	***				
Cu$_{TOT}$	-0.66	-1.382	0.18					
Time 28	**-31.62**	**-2.9**	**0.010**	*				
Time 88	**-22.69**	**-2.2**	**0.04**	*				
Location 2	**-17.88**	**-2.8**	**0.01**	*				
Time 28: Location 2	0.18	0.26	0.79					
Time 88: Location 2	0.87	1.35	0.19					

Deep								
ln(AVS) ~ Cu_{total} * Time + Location					6 and 22	37.24	0.93	< 0.0001
	Coeffic.	t- value	p - value					
Alpha	**2.18**	**6.634**	**1.14e^{-6}**	***				
Cu_{TOT}	**-0.16**	**-8.888**	**9.85e^{-9}**	***				
Day 28	-0.15	-0.362	0.721					
Day 88	-0.43	-1.006	0.326					
LM	0.17	0.768	0.450					
Cu_{TOT}:Day 28	-0.006	-0.245	0.809					
Cu_{TOT}:Day 88	0.013	0.506	0.618					
Cu_{SEM} ~ Cu_{total} * Time + Location					6 and 22	29.67	0.89	< 0.0001
	Coeffic.	t- value	p - value					
Alpha	-1.02	-0.664	0.5135					
Cu_{TOT}	**0.72**	**8.33**	**3.02e-8**	***				
Day 28	1.44	0.69	0.49					
Day 88	-0.500	-0.25	0.81					
LM	-1.66	-1.54	0.14					
Cu_{TOT}:Day 28	**-0.28**	**-2.35**	**0.03**	*				
Cu_{TOT}:Day 88	0.04	0.35	0.73					
$Cu_{ascorbate}$ ~ Cu_{total} * Time + Location					6 and 22	50.04	0.93	< 0.0001
	Coeffic.	t- value	p - value					
Alpha	**0.04**	**4.570**	**0.00015**	***				
Cu_{TOT}	**0.004**	**9.347**	**4.07e-9**	***				
Day 28	0.01	0.954	0.35					
Day 88	0.003	0.230	0.82					
LM	-0.01	-1.763	0.09	.				
Cu_{TOT}:Day 28	-0.0002	-0.301	0.766					
Cu_{TOT}:Day 88	0.0009	1.298	0.208					
Fe_{SEM} ~ Cu_{total} * Time + Location					6 and 22	1.72	0.32	0.16
Mn_{SEM} ~ Cu_{total} * Time + Location					6 and 22	1.013	0.21	0.44
FeO_x+MnO_x~ Cu_{total} * Time + Location					6 and 22	0.67	0.15	0.67

Table A4: Multiple regression analysis (forward stepping) of macroinvertebrate colonization in response to copper spiked sediments 4 weeks *in situ* aging. Models were selected from forward stepping procedure once all significant parameters were added. Final models are labeled in bold. Where location was included an interaction term they are designated by colon and as factor by River. Parameters are listed in the order which they were added to the models. n=10

	df	F-value	r^2	p-value
Density				
α + Cu_{SEM} (surface)	**1 and 8**	**47.34**	**0.98**	**< 0.001**
α				
Taxa				
α (Cu_{SEM} -AVS)/f_{oc} (surface):River + River + (Cu_{SEM} -AVS)/f_{oc} (surface)	**3 and 6**	**13.65**	**0.87**	**0.0043**
α + (Cu_{SEM} -AVS)/f_{oc} (surface):River + River	3 and 6	13.65	0.87	0.0043
α + (Cu_{SEM}-AVS)/f_{oc} (surface):River	2 and 7	12.41	0.78	0.005
α				
Chironomid Density				
α + Cu_{SEM} (surface) + Fe_{SEM} (deep)	**2 and 7**	**43.11**	**0.92**	**0.0001**
α + Cu_{SEM} (surface)	1 and 8	32.37	0.80	0.0005
α				
% Chironomids				
α + AVS (surface):River + Fe_{SEM} (deep):River	**4 and 5**	**24.05**	**0.95**	**0.001**
α + Fe_{SEM} (deep):River	2 and 7	5.653	0.62	0.03
α				
EPT Density				
α + $Cu_{ascorbate}$ (deep):River	**2 and 7**	**21.35**	**0..86**	**0.001**
α				
% EPT				
α + River	**1 and 8**	**27.37**	**0.77**	**0.0008**
α				
EPT Taxa				
α + $Cu_{ascorbate}$ (deep):River	**2 and 7**	**7.752**	**0.69**	**0.02**
α				
Simpson's D (1 – D)				
α + River	**1 and 8**	**19.07**	**0.70**	**0.002**
α				
Amphipod Density				
α + Cu_{SEM} (deep)	**1 and 8**	**17.4**	**0.68**	**0.003**
α				
% Amphipods				
α + $Cu_{ascorbate}$ (deep):River + $Cu_{ascorbate}$ (deep)	**2 and 7**	**8.947**	**0.72**	**0.01**
α + $Cu_{ascorbate}$ (deep):River	2 and 7	8.947		0.01
α				

Table A5: Multiple regression analysis (forward stepping) of macroinvertebrate colonization in response to copper spiked sediments after 12 weeks *in situ* aging. Models were selected from forward stepping procedure once all significant parameters were added. Final models are labeled in bold. Where location was included an interaction term they are designated by colon and as factor by River. Parameters are listed in the order which they were added to the models. n=10

		df	F-value	r^2	p-value
Density					
	α + Cu_{total} (surface) + FeO_x+MnO_x (deep):River + FeO_x+MnO_x (deep)	**3 and 6**	**31.65**	**0.64**	**0.03**
	α + Cu_{total} (surface) + FeO_x+MnO_x (deep):River	3 and 6	31.65	0.64	0.03
	α + Cu_{total} (surface)	1 and 8	15.56		
	α				
Taxa					
	α + (Cu_{SEM} -AVS)/f_{oc} (deep)	**1 and 8**	**6.481**	**0.45**	**0.03**
	α				
% Chironomid					
	α + Cu_{SEM} –AVS (deep)	**1 and 8**	**9.306**	**0.54**	**0.02**
	α				
Chironomid Density					
	α				
EPT Density					
	α				
% EPT					
	α				
EPT Taxa					
	α + River	1 and 8	5.556	**0.41**	0.05
	α				
Simpson's D (1 – D)					
	α + CU_{SEM} –AVS (deep) + Cu_{total} (deep):RIVER	3 and 6	14.23	**0.64**	0.006
	α + CU_{SEM} –AVS (deep)	1 and 8	10.67	0.57	0.01
	α				
Amphipod Density					
	α + FeO_x+MnO_x (deep):River + FeO_x+MnO_x (deep)	**2 and 7**	**17.08**	**0.83**	**0.002**
	α + FeO_x+MnO_x (deep):River	2 and 7	17.08	0.83	0.002
	α				
% Amphipods					
	α + Fe_{SEM} (surface):River + Fe_{SEM} (surface)	**2 and 7**	**13.68**	**0.80**	**0.004**
	α + Fe_{SEM} (surface):River	2 and 7	13.68	0.80	0.004
	α				

Table A6: Macroinvertebrate colonization of copper spiked sediments (Raisin) in Pine and Little Molasses Rivers. Cu represents nominal copper values.

			Abundance (No. m^{-2})				Simpson	Richness (No.)		Relative abundance (%)		
	Wk.	Cu	Ab.	amphipod	Chiron.	EPT	(1-D)	Taxa	EPT	EPT	Amphipods	Chiron.
Pine	1	0	1024	79	472	394	0.78	5	2	46.15	7.69	38.46
		380	1181	0	709	472	0.76	4	3	60.00	0.00	40.00
		750	394	0	315	79	0.70	3	2	80.00	0.00	20.00
		1200	1496	0	1024	394	0.84	8	6	68.42	0.00	26.32
		2100	866	0	394	236	0.87	6	3	45.45	0.00	27.27
	4	0	5039	236	1339	2677	0.69	10	5	26.56	4.69	53.13
		380	2441	0	866	1102	0.71	6	2	35.48	0.00	45.16
		750	2047	79	472	866	0.78	7	2	23.08	3.85	42.31
		1200	1260	0	315	472	0.83	6	2	25.00	0.00	37.50
		2100	2047	0	709	551	0.83	8	4	34.62	0.00	26.92
	12	0	4567	79	79	3228	0.47	7	1	1.72	1.72	70.69
		380	1890	79	394	551	0.87	10	3	20.83	4.17	29.17
		750	2441	0	157	1732	0.48	6	2	6.45	0.00	70.97
		1200	2047	157	236	1260	0.62	7	2	11.54	7.69	61.54
		2100	1654	79	79	1260	0.42	5	1	4.76	4.76	76.19
Little Molasses	1	0	2362	315	236	1811	0.40	3	1	10.00	13.33	76.67
		380	2205	394	157	1654	0.42	3	1	7.14	17.86	75.00
		750	1890	315	394	1102	0.61	4	1	20.83	16.67	58.33
		1200	2126	236	79	1732	0.33	4	1	3.70	11.11	81.48
		2100	945	0	79	866	0.17	2	1	8.33	0.00	91.67
	4	0	8740	5591	236	2520	0.51	6	2	2.70	63.96	28.83
		380	5748	2520	157	2835	0.57	5	1	2.74	43.84	49.32
		750	2047	1102	0	394	0.64	4	0	0.00	53.85	19.23
		1200	394	0	79	315	0.40	2	1	20.00	0.00	80.00
		2100	472	0	0	394	0.33	2	0	0.00	0.00	83.33
	12	0	2126	315	79	945	0.75	7	1	3.70	14.81	44.44
		380	1496	551	157	315	0.81	6	1	10.53	36.84	21.05
		750	2756	1417	157	1024	0.61	4	1	5.71	51.43	37.14
		1200	2992	1732	79	1024	0.56	5	1	2.63	57.89	34.21
		2100	945	0	0	866	0.17	2	0	0.00	0.00	91.67

Table A7: Macroinvertebrate colonization of field contaminated sediments (Ocoee) in Pine and Little Molasses Rivers. Cu represents nominal copper values.

			Abundance (No. m^{-2})				Simpson	Richness (No.)		Relative abundance (%)		
	Wk.	Cu	Ab.	amphipod	Chiron.	EPT	(1-D)	Taxa	EPT	EPT	Amphipods	Chiron.
Pine	1	170	157	0.00	0	157	0	1	100.00	0.00	0.00	1
		600	709	0.64	0	315	394	3	44.44	0.00	55.56	2
		1250	551	0.86	0	157	79	4	28.57	0.00	14.29	1
		1600	630	0.82	0	394	157	4	62.50	0.00	25.00	2
	4	170	1969	0.89	79	472	472	10	24.00	4.00	24.00	3
		600	157	1.00	0	79	0	2	50.00	0.00	0.00	1
		1250	394	1.00	0	79	79	5	20.00	0.00	20.00	1
		1600	1339	0.81	0	630	236	7	47.06	0.00	17.65	2
	12	170	1260	0.84	0	157	157	7	12.50	0.00	12.50	1
		600	79	1.00	0	0	79	1	0.00	0.00	100.00	0
		1250	1024	0.60	0	0	394	3	0.00	0.00	38.46	0
		1600	1102	0.75	0	236	551	6	21.43	0.00	50.00	2
Little Molasses	1	170	866	0.56	236	0	551	3	0.00	27.27	63.64	0
		600	1102	0.14	79	0	1024	2	0.00	7.14	92.86	0
		1250	945	0.45	79	79	709	4	8.33	8.33	75.00	1
		1600	1181	0.47	157	79	866	4	6.67	13.33	73.33	1
	4	170	7323	0.59	4252	157	1890	6	2.15	58.06	25.81	2
		600	2205	0.42	1654	0	394	3	0.00	75.00	17.86	0
		1250	6063	0.54	2126	79	3543	5	1.30	35.06	58.44	1
		1600	1496	0.62	630	157	709	3	10.53	42.11	47.37	1
	12	170	1181	0.54	787	79	79	4	6.67	66.67	6.67	1
		600	394	0.80	0	0	157	3	0.00	0.00	40.00	0
		1250	1417	0.77	630	0	236	6	0.00	44.44	16.67	0
		1600	1102	0.79	472	236	236	6	21.43	42.86	21.43	2

Table A8: Sediment geochemical characteristics of Cu spiked surface sediments (Raisin) in Pine and Little Molasses Rivers.

	Wk	Cu	OC	AVS	Cu_{total}	Cu_{SEM}	$Cu_{ascbt.}$	Cu_{SEM}-AVS	FeO_x+MnO_x	Cu_{SEM}-AVS / f_{OC}
			(%)	(µmol g^{-1} dw)						(µmol g_{oc}^{-1})
Pine	1	0	0.03	11.47	0.05	0.07	0.07	-11.41	78.16	-356.14
		380	0.03	1.95	6.01	3.33	0.10	1.38	73.41	45.58
		750	0.03	2.49	10.63	4.23	0.09	1.74	74.19	62.39
		1200	0.03	0.99	16.20	10.25	0.17	9.26	71.46	286.19
		2100	0.03	0.05	26.02	23.92	0.26	23.87	76.12	840.16
	4	0	0.03	6.65	0.22	0.22	0.07	-6.54	64.60	-223.66
		380	0.03	1.76	5.76	2.37	0.06	3.77	107.35	144.20
		750	0.03	1.70	12.72	5.52	0.08	0.67	68.78	23.20
		1200	0.02	0.25	13.61	7.18	0.11	6.92	75.64	323.71
		2100	0.03	0.04	34.14	18.10	0.23	18.05	64.19	608.96
	12	0	0.03	3.52	0.01	0.09	0.04	-5.08	68.94	-157.69
		380	0.03	1.04	4.52	3.21	0.08	2.17	74.26	86.95
		750	0.03	0.73	8.48	5.94	0.10	5.20	70.59	170.78
		1200	0.03	0.20	18.78	7.67	0.18	7.47	61.08	273.33
		2100	0.03	0.10	30.89	25.52	0.39	25.42	69.24	811.27
Little Molasses	1	0	0.03	10.90	0.10	0.15	0.04	-10.75	71.80	-344.51
		380	0.03	3.56	5.67	2.49	0.08	-1.06	60.58	-36.18
		750	0.03	1.17	13.09	8.47	0.09	7.30	64.07	250.48
		1200	0.03	0.27	16.58	9.60	0.09	9.33	58.61	328.32
		2100	0.03	0.07	28.50	19.15	0.16	19.09	75.44	678.93
	4	0	0.01	8.47	0.15	0.04	0.03	-8.43	62.95	-1005.51
		380	0.01	0.13	0.36	0.32	0.04	0.19	78.86	31.27
		750	0.01	0.68	5.83	4.68	0.08	4.00	64.45	286.83
		1200	0.01	0.16	2.53	3.01	0.09	2.86	59.80	567.70
		2100	0.01	0.08	9.63	15.51	0.13	15.43	63.82	1447.64
	12	0	0.01	7.24	0.11	0.04	0.03	-7.20	75.61	-674.12
		380	0.02	2.57	4.92	1.19	0.07	-1.38	76.78	-58.60
		750	0.01	0.73	1.48	3.62	0.05	2.89	57.36	506.61
		1200	0.004	0.18	1.06	4.03	0.05	3.85	54.04	863.69
		2100	0.02	0.08	28.10	20.12	0.23	20.03	71.95	805.11

Table A9: Sediment geochemical characteristics of Cu spiked deep sediments (Raisin) in Pine and Little Molasses Rivers.

	Wk	Cu	OC (%)	AVS	Cu_{total}	Cu_{SEM}	$Cu_{ascbt.}$	Cu_{SEM}-AVS	FeO_x+MnO_x	Cu_{SEM}-AVS /f_{OC} (μmol g_{oc}^{-1})
				(μmol g^{-1} dw)						
Pine	1	0	0.03	11.44	0.01	0.03	0.05	-11.40	78.16	-367.04
		380	0.03	4.62	5.52	2.20	0.06	-2.42	73.41	-79.38
		750	0.03	1.15	11.32	6.32	0.08	-1.02	74.19	-33.87
		1200	0.03	0.38	17.66	10.22	0.10	9.84	71.46	317.68
		2100	0.03	0.12	28.05	26.28	0.21	26.16	76.12	856.99
	4	0	0.03	12.08	0.11	0.12	0.04	-11.96	64.60	-383.64
		380	0.03	2.44	6.75	2.25	0.10	-0.19	107.35	-6.97
		750	0.03	1.44	14.03	2.65	0.10	1.21	68.78	43.98
		1200	0.03	0.17	21.08	11.30	0.14	11.13	75.64	383.27
		2100	0.03	0.03	29.04	14.77	0.18	14.74	64.19	490.72
	12	0	0.04	9.91	0.01	0.03	0.04	-9.88	68.94	-279.11
		380	0.03	1.94	5.90	0.99	0.06	-0.95	74.26	-32.53
		750	0.03	0.78	8.72	4.43	0.09	3.65	70.59	145.17
		1200	0.03	0.24	12.89	5.97	0.10	5.73	61.08	193.05
		2100	0.03	0.08	35.40	24.72	0.23	24.64	69.24	870.68
Little Molasses	1	0	0.03	8.59	0.18	0.07	0.04	-8.52	71.80	-279.37
		380	0.03	4.33	7.51	2.18	0.06	-2.15	60.58	-77.05
		750	0.03	0.98	12.19	3.99	0.07	3.01	64.07	100.20
		1200	0.03	0.58	17.15	6.56	0.09	5.97	58.61	210.55
		2100	0.03	0.04	33.91	19.10	0.17	19.05	75.44	689.62
	4	0	0.03	6.97	0.00	0.00	0.03	-6.97	62.95	-248.02
		380	0.02	3.81	5.02	2.10	0.08	-1.72	78.86	-77.06
		750	0.03	1.19	13.57	3.82	0.09	2.62	64.45	101.73
		1200	0.02	0.40	12.60	5.60	0.10	5.20	59.80	283.74
		2100	0.02	0.06	33.90	12.41	0.18	12.35	63.82	536.45
	12	0	0.03	12.67	0.00	0.07	0.03	-12.60	75.61	-412.99
		380	0.03	4.12	6.57	1.09	0.09	-3.03	76.78	-100.35
		750	0.03	1.75	12.12	1.73	0.07	-0.02	57.36	-0.69
		1200	0.01	0.34	8.24	3.36	0.10	3.02	54.04	203.01
		2100	0.03	0.07	24.74	20.71	0.18	20.64	71.95	753.77

Table A10: Sediment geochemical characteristics of field contaminated surface sediments (Ocoee) in Pine and Little Molasses Rivers.

	Wk.	Cu	OC	AVS	Cu$_{total}$	Cu$_{SEM}$	Cu$_{ascbt.}$	Cu$_{SEM}$-AVS	FeO$_x$+MnO$_x$	Cu$_{SEM}$-AVS /f_{OC}
			%	(μmol g^{-1} dw)						(μmol g$_{oc}$$^{-1}$)
Pine	1	170	0.01	0.01	4.05	0.68	0.33	-0.18	26.25	-34.15
		600	0.02	3.89	9.65	1.48	0.04	-2.89	41.62	-178.70
		1250	0.02	0.00	27.29	7.91	0.22	4.43	54.90	257.24
		1600	0.01	7.98	29.53	2.83	0.03	-3.46	50.03	-248.89
	4	170	0.01	0.35	4.76	0.82	0.08	-0.36	22.87	-38.78
		600	0.02	3.83	9.53	1.57	0.01	-2.70	40.87	-128.63
		1250	0.02	0.08	29.68	12.82	0.16	4.24	54.42	230.55
		1600	0.02	7.50	30.60	2.74	0.01	-3.49	66.62	-208.51
	12	170	0.01	0.02	3.30	0.49	0.21	-0.53	24.65	-81.37
		600	0.02	3.20	8.16	1.24	0.01	-2.34	34.05	-128.23
		1250	0.02	0.01	29.99	9.15	0.20	4.08	41.23	241.62
		1600	0.01	6.49	30.92	3.10	0.01	-2.83	46.77	-219.25
Little Molasses	1	170	0.01	0.00	4.54	0.74	0.32	-0.10	25.54	-16.19
		600	0.02	2.88	10.24	0.90	0.03	-2.65	33.16	-159.55
		1250	0.02	0.01	29.15	7.20	0.24	4.60	47.08	255.31
		1600	0.01	9.21	29.99	2.86	0.42	-6.47	68.90	-437.92
	4	170	0.01	0.02	2.84	0.55	0.15	-0.45	21.29	-84.95
		600	0.02	3.35	7.45	0.90	0.01	-2.95	34.26	-195.86
		1250	0.02	0.01	22.53	7.12	0.18	3.61	51.45	203.07
		1600	0.19	14.53	20.99	3.49	0.01	-11.45	60.34	-59.17
	12	170	0.01	0.37	3.33	0.44	0.06	-1.06	13.95	-121.47
		600	0.02	2.17	7.09	1.05	0.01	-1.63	32.85	-87.39
		1250	0.02	0.01	19.34	5.56	0.19	2.08	40.13	128.21
		1600	0.01	6.42	23.08	6.37	0.03	-2.23	30.33	-153.43

Table A11: Sediment geochemical characteristics of field contaminated deep sediments (Ocoee) in Pine and Little Molasses Rivers.

	Wk.	Cu	OC	AVS	Cu_{total}	Cu_{SEM}	$\text{Cu}_{\text{ascbt.}}$	Cu_{SEM}-AVS	FeO_x+MnO_x	Cu_{SEM}-AVS /f_{OC}
			%	(μmol g^{-1} dw)						(μmol g_{oc}^{-1})
Pine	1	170	0.01	0.00	4.48	0.61	0.20	0.80	26.25	114.23
		600	0.02	2.42	9.88	2.24	0.04	0.67	41.62	-10.79
		1250	0.02	0.01	26.04	4.94	0.22	6.54	54.90	284.38
		1600	0.01	9.01	29.98	3.33	0.04	-2.86	50.03	-401.37
	4	170	0.01	0.02	4.52	0.42	0.13	0.57	22.87	67.05
		600	0.02	1.84	8.81	1.38	0.01	0.09	40.87	-21.51
		1250	0.02	0.02	27.83	6.03	0.21	7.77	54.42	285.65
		1600	0.02	8.74	28.02	2.19	0.01	-3.59	66.62	-401.18
	12	170	0.01	0.02	3.14	0.46	0.12	0.63	24.65	81.88
		600	0.02	2.16	7.75	1.30	0.01	-0.27	34.05	-45.12
		1250	0.02	0.01	24.86	6.96	0.20	8.73	41.23	397.87
		1600	0.01	8.51	29.26	3.67	0.01	-2.10	46.77	-369.37
Little Molasses	1	170	0.01	0.01	4.03	0.64	0.18	0.84	25.54	108.37
		600	0.02	4.11	9.35	0.77	0.03	-2.87	33.16	-198.46
		1250	0.02	0.01	25.97	7.25	0.03	9.23	47.08	389.23
		1600	0.02	9.92	31.38	2.47	0.03	-6.00	68.90	-491.81
	4	170	0.01	0.02	2.87	0.48	0.13	0.67	21.29	76.75
		600	0.01	3.91	7.73	0.85	0.01	-2.54	34.26	-209.88
		1250	0.02	0.01	24.19	8.34	0.21	10.21	51.45	479.13
		1600	0.01	7.38	25.60	2.75	0.01	-2.33	60.34	-333.50
	12	170	0.01	0.01	3.02	0.35	0.08	0.48	13.95	58.74
		600	0.02	2.65	6.57	0.76	0.01	-0.33	32.85	-112.90
		1250	0.02	0.02	25.58	6.56	0.01	8.66	40.13	420.40
		1600	0.02	7.79	21.66	2.39	0.07	-3.82	30.33	-306.07