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Association between extracted copper and dissolved organic matter in dairy-manure amended soils☆

Eloá Araújo [a], Daniel G. Strawn [b, *], Matthew Morra [b], Amber Moore [c], Luis Reynaldo Ferracciú Alleoni [a]

[a] Soil Science Dep., P.O. Box 09, Univ. of Sao Paulo, Piracicaba, SP, 13418-900, Brazil
[b] 875 Perimeter Dr. MS 2340, University of Idaho, Moscow, ID, 83844-2340, USA
[c] Agricultural & Life Sciences 3063, 2750 SW Campus Way, Corvallis, OR, 97331, USA

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ABSTRACT

Dairy manure often has elevated concentrations of copper (Cu) that when applied to soil may create toxicity risks to seedlings and soil microbes. Manure application also increases dissolved organic matter (DOM) in soil solution. We hypothesize that high rates of dairy manure amendment over several years will cause increased DOM in the soil that complexes Cu, increasing its mobility. To test this hypothesis, this study investigated water soluble Cu concentrations and dissolved organic carbon (DOC) in soil samples from 3 years of manure-amended soils. Samples were collected at two depths over the first 3 years of a long-term manure-amendment field trial. DOC, Cu, Fe, and P concentrations were measured in water extracts from the samples. Ultraviolet/visible (UV/Vis) spectra were used to assess the DOC characteristics. After 3 years of manure application, extractable Cu concentration was approximately four times greater in the surface and two times greater in subsurface samples of manure-amended soils as compared to non-amended control soils and traditional mineral fertilizer-amended soils. The extractable Cu concentration was greatest in plots that had the highest manure amendment rates (35 t ha^{-1} and 52 t ha^{-1}, dry weight). The UV/Vis parameters $SUVA_{254}$ and E_2/E_3 correlated with Cu concentration in the extracts ($p < 0.05$), suggesting that DOC characteristics are important in Cu-binding. The molecular characteristics of the DOC in the subsurface after 3 years of manure amendment were distinct from the DOC in the control plot, suggesting that manure amendment creates mobile DOC that may facilitate Cu mobilization through soil. The 10-fold increase in extractable Cu concentration after only 3 years of manure application indicates that repeated applications of the dairy manure sources used in this study at rates of 35 t/ha or greater may create risks for Cu toxicity and leaching of Cu into ground and surface waters.

1. Introduction

Copper (Cu) is a micronutrient needed in trace amounts by plants. In soils, however, elevated Cu concentrations can be toxic, negatively impacting seed germination and soil microorganisms. Dairy manure added to soils to increase organic matter and nutrients is a source of Cu. There are two main routes of Cu introduction into dairy manure: i) Cu added to hoof baths at 5–10% concentrations to control the incidence of hoof disorders commonly comingles with manure when barn floors are washed (Ippolito et al., 2013; Osorio et al., 2016); and ii) Cu is fed to dairy cows as a micronutrient, of which a majority is excreted by the cows in their manure (Hristov et al., 2006). Because of the elevated content of Cu in manure, manure application to soils creates concern for impairment of soil health and potential leaching of Cu out of the soils into irrigation return water or shallow groundwater where it may impair water quality.

Manure application increases soil organic matter and nutrient concentrations. Higher amounts of soil organic matter increase concentrations of dissolved organic carbon (DOC). Dissolved organic carbon, a product of plant and microbial residue decomposition, plays an important role in many biogeochemical processes

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* Corresponding author.
E-mail addresses: eloama@hotmail.com (E. Araújo), dgstrawn@uidaho.edu (D.G. Strawn), mmorra@uidaho.edu (M. Morra), amber.moore@oregonstate.edu (A. Moore), alleoni@usp.br (L.R. Ferracciú Alleoni).

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(Antoniadis and Alloway, 2002; Hagedorn et al., 2015). Knowledge of the molecular properties of DOC is useful to infer reactivity and mobility of soil organic materials, as well as its reactivity with other chemicals within soil. DOC can complex Cu, changing its mobility and availability. Ashworth and Alloway (2004) observed that application of sewage sludge to a sandy loam soil column resulted in a positive correlation between DOC and Cu that facilitated Cu mobilization through the column. Zhang and Zhang (2010) observed that phosphorus (P) application increased leaching of Cu through packed soil columns amended with ammonium phosphate. They attributed this to competitive sorption between DOC and P for soil sorption sites. The interaction of Cu, DOC and P is relevant to dairy-manure amended soils because manure contains high concentrations of these chemicals.

To evaluate DOC characteristics, spectroscopic techniques are used to probe molecular composition and reactivity (Chen et al., 2016). Ultraviolet/visible (UV/Vis) spectroscopy is a rapid, high sensitivity technique that allows for investigation of the composition of natural organic matter (Hassouna et al., 2010). Weishaar et al. (2003) showed that the specific UV absorbance measured at 254 nm ($SUVA_{254}$) strongly correlates with the degree of aromaticity of DOC, which was verified by ^{13}C NMR (nuclear magnetic resonance). A second UV–Vis parameter used to characterize organic matter is the ratio of absorbance at 250 nm to the absorbance at 365 nm, defined as the E_2/E_3 ratio, which correlates to the molecular size of DOC molecules (Thomsen et al., 2002). Aromaticity ($SUVA_{254}$) has been related to increases in the E2/E3 ratio, and therefore molecular size (Thomsen et al., 2002; Mouloubou et al., 2016).

Complexation of metals by DOC enhances metal mobility through soil profiles (Weng et al., 2002; Jansen et al., 2003; Zhang and Zhang, 2010; Ren et al., 2015; Li et al., 2016). At typical soil pH, DOC has a net negative charge, making it mobile in negatively charged soils (Ashworth and Alloway, 2004). Hagedorn et al. (2015) used autoradiographs to monitor DOC migration through a soil profile and showed that dissolved organic ^{14}C ($DO^{14}C$) was retained in the upper 3-cm of soil columns, but in deeper soil, ^{14}C was concentrated along soil pores and textural discontinuities. Their results suggested that in well-structured soils, DOC is transported through preferential flow pathways. Hagedorn et al. (2015) also showed that leaching of $DO^{14}C$ correlated negatively with oxalate-extractable Al, Fe, and Mn, suggesting that soil oxides can immobilize DOC. Thus, in soils with high amounts of oxide minerals, DOC mobilization may be inhibited. Potential toxicity risks of Cu in surface waters is assessed using the biotic-ligand model (U.S. Environmental Protection Agency, 2007), which includes complexation of Cu with ligands in the toxicity assessment. DOC is one of the most common metal complexing ligands in natural waters. The agreement between this well-established Cu bioavailability model and aquatic organism toxicity confirms that DOC is an important component in solutions with respect to Cu mobilization. DOC-Cu complexation may be especially important in manure-amended soils given the high amounts of soluble organic matter in manure.

Increases in DOC concentration in soil solutions from animal manure amendment have been studied. Rochette and Gregorich (1998) observed that dairy manure addition at 100 t ha^{-1} (wet weight) to an acidic soil increased soluble organic carbon by approximately three times compared to a control soil. Sun et al. (2017) studied Cu in pig manure-amended acidic soils amended for 23 years and observed increased bioavailability of Cu (>20 times) and increased DOC (~two times) in manure-amended soils. The fate of DOC and metals such as Cu applied in animal manure depends on manure and soil characteristics, as well as application rate and other agronomic factors, such as tillage, irrigation, and specific crop.

While many researchers have characterized soil organic matter complexation with Cu (e.g. (Temminghoff et al., 1997; Strobel et al., 2005; Karlsson et al., 2006),), and other researchers have monitored Cu-organic matter interactions in short-term Cu-spiked incubations (e.g. Brandt et al. (2008)), few studies have been carried out to characterize DOC-Cu mobilization in soils amended for multiple years. Sun et al. (2017), monitored Cu in a long-term pig manure amendment study on acidic soils. However, manure type and soil characteristics, such as pH, are important variables that affect chemical processes in manure-amended soils. Thus, in this study, we are investigating Cu solubility in dairy-manure amended alkaline soils over 3 years of annual dairy manure amendment at three different rates. Alkaline soils are common in semi-arid and arid regions such as the western United States where average dairy herd size is much greater than other milk producing regions in the US. Because of massive manure applications to soils near dairies, there is a need to understand how dairy manure amendments affect Cu availability in these alkaline soils. This study is one of the first to study Cu and DOC availability in diary-manure amended soils that are not spiked with Cu; the Cu is added to the soils by typical dairy industry practices.

We hypothesized that in alkali soils subjected to high manure application rates, increased Cu and DOC associations will promote the transport of Cu through the soil profile. To test this hypothesis, soluble Cu and DOC from soils subjected to annual manure application for 3 years were evaluated. Concentrations of DOC and Cu released from surface and subsurface soils by DI water were measured and UV spectroscopy was used to analyze molecular characteristics of DOC from the soils. Characterizing the long-term fate of Cu additions to soils from dairy manure amendments is important for maintaining soil health under intensive agricultural production practices.

2. Materials and methods

2.1. Sample characterization

The study plots are in a long-term manure application experiment in Kimberly, Idaho (United States) (42̊33′ N; 114̊21′ W). The soil is Portneuf silt loam, a coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid. Mean annual precipitation in the region is 22-cm and mean annual temperature is 9.6 °C. The experiment is designed in randomized blocks with four replicates, with each block consisting of all treatments. The manure is applied at two application frequencies (annual and biennial), and three yearly application rates (17 t ha^{-1}; 35 t ha^{-1}; 52 t ha^{-1} of dairy manure, on a dry weight basis). The 17 t ha^{-1} rate is representative of observed manure application rates for fields that are several kilometers from dairy operations, while the 52 t ha^{-1} rate is representative of manure application rates for fields that are within a kilometer of a dairy. Annual treatments received manure application in the fall of 2012, 2013, and 2014; biennial treatments received manure applications in the fall of 2012 and 2014. Fertilizer treatments (N, P, and K mineral fertilizers) were applied at recommended agronomic rates to one set of plots. A control plot with no applied nutrients was also established.

The applied manure had average (n = 3) pH of 8.8 (±0.17); 291.2 (±62.90) g kg^{-1} of total organic carbon; 63.9 (±12.12) mg kg^{-1} of total P; and 89.3 (±31.95) mg kg^{-1} of total Cu. Plots from three blocks were used for the laboratory experiments. The soils were sampled at 0–30 cm and 30–60 cm depths before planting of wheat (*Triticum aestivum* L) in 2013; potato (*Solanum tuberosum* L.) in 2014; and barley (*Hordeum vulgare* L.) in 2015. Average pH, P_{NaHCO3}, DTPA extractable Cu, and total organic matter content are provided for the unamended soil plots in Supplementary Material

Table S1. Total concentrations of Cu and P in the samples are provided in Supplementary Material Table S2.

2.2. DOC extraction and filtration

DOC was extracted from the soil and manure samples using 18 MΩ deionized water with a solid to solution ratio of 1:5 (g:ml). Preliminary kinetic experiments (data not shown) showed that desorbed Cu concentrations did not change after 2 h, suggesting equilibrium. The suspensions were shaken for 2 h on an end-over-end shaker at 10 rpm. Following mixing, the suspensions were centrifuged at 1500 rpm (526 g-force) for 7 min, and then filtered through a 45-mm diameter, 0.45-µm mixed cellulose membrane filter (Merck Millipore Ltd., Tullagreen, Ireland). After filtration, the extracts were stored and kept at 4 °C until they were analyzed.

2.3. Extract characterization

The DOC content was determined using a TOC analyzer (model TOC-L, Shimadzu, Maryland) for total organic carbon measurement after acidification to remove carbonates. Standardization using known TOC solutions and measurement of a blank spike were used to ensure accuracy of the TOC analyzer. The content of water extractable Ca, Cu, Fe, and P were determined by atomic emission spectrometry (ICP-OES, model Thermo-iCAP 6000, Waltham, Massachusetts), which was standardized using NIST traceable standards (Specpure Inc., Ward Hill, Massachusetts). Dissolved reactive P (DRP) was determined using a molybdate blue colorimetric test on the water extracts (Pote and Daniel, 2009), with solution absorption measured on a Thermo Scientific Genesys 10S UV–Vis spectrophotometer (Waltham, Massachusetts).

Using UV/Vis absorbance spectra can provide information on the chemical characteristics of DOC, including information on aromaticity, and molecular weight and size (He et al., 2011). UV spectra were measured on a Thermo Scientific Genesys 10S UV–Vis spectrophotometer using 1-cm path-length disposable plastic cuvettes (Brandtech Inc., Essex, Connecticut) in the wavelength range of 200–700 nm. The cuvettes are made of a proprietary polycyclical olefin that is UV-transparent, and thus allow for accurate collection of UV spectra down to 230 nm. The specific UV absorbance at 254 nm ($SUVA_{254}$ ($L\ mg^{-1}\ cm^{-1}$)) was used as an aromaticity index by normalizing the absorbance at 254 nm of each sample by the respective DOC concentration ($mg\ L^{-1}$) (Mouloubou et al., 2016). The E_2/E_3 ratio (the absorbance at 250 nm divided by the absorbance at 365 nm) was calculated to assess the molecular size index (Thomsen et al., 2002).

2.4. Data analysis

The statistical software SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to compare means using least square means and Tukey mean separation tests ($p < 0.05$). Pearson's Correlation tests were performed to investigate relationships between variables.

3. Results and discussion

3.1. Extractable Cu and DOC concentrations

In surface soils, annual application of manure for 3 years caused an increase in water extractable Cu concentrations in all the manure treatments except for the 17 t ha^{-1} biennial application (Fig. 1). In the subsurface soil samples, a significant increase in water extractable Cu occurred only after 3 years of the two highest manure application rates (52 and 35 t ha^{-1} annual and 52 t ha^{-1} biennial). The extractable Cu concentration was at maximum 1.3%

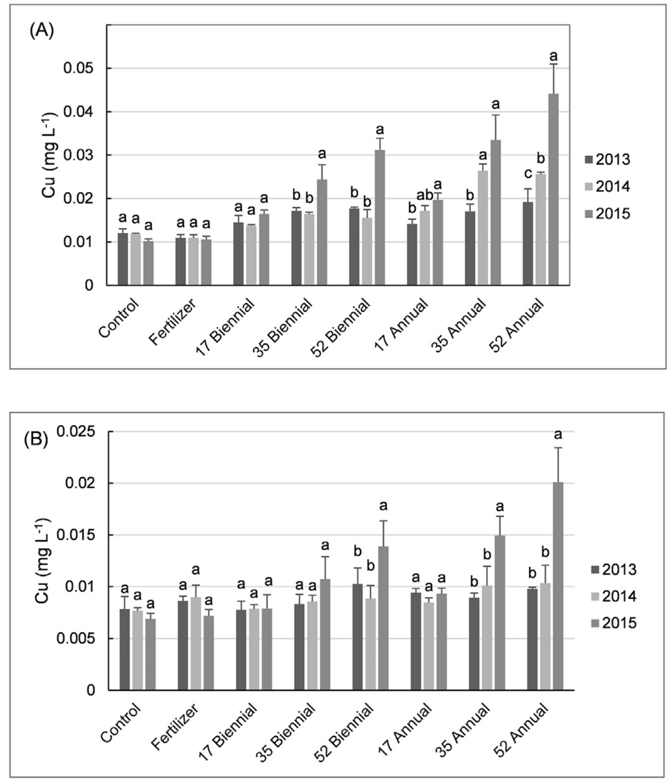

Fig. 1. Mean Cu concentration in soil extracts from the soil samples collected from 0 to 30 cm (A) and 30–60 cm (B) in 2013, 2014, and 2015. Numbers preceding the x-axis labels indicate application rate in t ha^{-1}. Different letters represent statistical difference by least square means tests with measures repeated on the year for each treatment ($p < 0.05$). Error bars indicate standard error of mean.

of the total soil Cu (2015 52 t ha^{-1} annual application sample, See Table S2 in Supplementary Material) indicating that only a small fraction of the total Cu in the soil is soluble. This suggests that a stock of soil Cu exists that could be slowly leached from the soil for many years, even after addtion of Cu-containing manure stops.

After 3 years of manure amendment, extractable Cu concentrations in the two highest manure application rate treatments were about four times higher than Cu concentrations in the control and fertilizer plots. In the first year, the 17 t ha^{-1} annual and biennial treatments had 1.5 times lower extractable Cu concentration than the 52 t ha^{-1} manure application, but twice the extractable Cu concentration of the control samples. Water extractable Cu concentration from the manure itself averaged 2.85 (±1.24) mg L^{-1}, suggesting that manure is the source for increased extractable Cu in the soils. Sun et al. (2017) studied agricultural soils under long-term pig manure application to acidic soils and observed that increases in Cu concentration in soil caused increased bioavailability in the form of elevated Cu concentrations in wheat straw and corn (*Zea mays* L.) stalks.

In the 35 and 52 t ha^{-1} samples, annual application of manure led to increased extractable Cu concentrations at the 0–30 cm soil depth compared to the other treatments (Fig. 1 and Supplementary Table S3). After 3 years, the 52 t ha^{-1} biennial application samples had similar extractable Cu concentrations as extracts from the 35 t ha^{-1} annual application, suggesting that multiple high application rates of manure cause extractable Cu concentrations to dramatically increase, even if applied only biennially.

Water extracted Cu concentrations are a good measure of concentrations of the element in soil pore-water, and thus bioavailability. Moreno-Jiménez et al. (2011) compared the phytotoxicity of metals in soil solution extracted from soil pore water and deionized water soil extractions (1:20 soil:solution ratio) and found that both solutions reduced germination of *Lolium perenne* seeds. In addition to phytotoxicity, if Cu is leached out of the soil, elevated Cu concentrations in soil pore water pose potential risks to water quality. The total soil Cu concentrations, 12–17 mg kg^{-1} (Supplementary Material Table S2), are within the range of typical uncontaminated soils (2–250 mg kg^{-1}) (Bowen, 1979). While there are no listed limits for soil water, typical freshwater concentrations of Cu are 0.003 mg L^{-1} (Bowen, 1979), and the maximum contaminant level goal for Cu in drinking water is 1.3 mg L^{-1} (EPA, 2009). Copper concentrations in soil extracts in this study (Fig. 1) are approximately ten times greater than present in uncontaminated freshwater ecosystems. A 10-fold increase in extractable Cu concentration from the soils after only 3 years of manure application suggests that high manure application rates may increase dissolved Cu concentrations sufficiently to affect soil quality and plant growth (Rooney et al., 2006). The potential risks of elevated Cu concentrations to aquatic ecosystems by way of leaching or runoff are also likely to increase.

3.2. Extractable DOC

Extractable DOC concentrations increased with manure application rates and number of applications (Fig. 2 and Supplementary Material Table S3). DOC concentration decreased with soil depth in all treatments. In the 35 t ha^{-1} and 52 t ha^{-1} annual manure application rate treatments, subsurface DOC content was less than half of surface DOC content. Elevated DOC concentrations in upper soil layers are related to both increased input from plant residues (Hassouna et al., 2010), organic amendments such as manure, and their respective degradation by-products. DOC in the deeper layers is proposed to be composed of aged compounds, lixiviated organic compounds, or resynthesized carbon compounds from microbial activity (von Lutzow et al., 2006; Kaiser and Kalbitz, 2012).

The Cu/DOC ratio (Supplementary Material Figure S1) can be used as an indicator of Cu-DOC binding because it allows for a relative comparison of the affinity and association between the dissolved metal and organic matter (Nierop et al., 2002). Fig. 3 shows extractable Cu concentrations versus DOC for all the plots and years. There is an overall positive linear correlation for the combined data ($r^2 = 0.925$, $p < 0.01$); however, the presence of two distinct relationships suggests that extractable Cu-DOC interactions differ (Fig. 3). The year 2015 0–30 cm samples had a greater Cu/DOC ratio than the 2014 or 2013 samples, indicating that the highest rate of manure input caused an increase in extractable Cu concentration without a corresponding increase in extractable DOC concentration. In subsurface soils, after 3 years of cumulative annual application, the Cu/DOC relationship of the three samples with the highest manure input rates were distinct from the other samples (Fig. 3; Supplementary Figure S1).

At low Cu^{2+} activity, high affinity sites on DOC are preferentially occupied by Cu (Amery et al., 2010). As Cu^{2+} activity increases, low affinity sites become occupied. Thus, high Cu/DOC ratios suggest that Cu is occupying high and low affinity sites. In the 52 t ha^{-1} annual manure input soil extracts, the Cu/DOC ratios in samples from both depths are similar (Supplementary Material Figure S1), suggesting that at high manure application rates, a strong Cu-DOC association occurs even in lower soil layers. This suggests that increased manure application is promoting Cu-DOC complexation and consequently increasing Cu mobility because Cu associated with DOC extract is more soluble and mobile than non-complexed Cu.

3.3. Relationship between Extractable Cu, DOC, P and Fe

There was a strong positive correlation between Cu, total

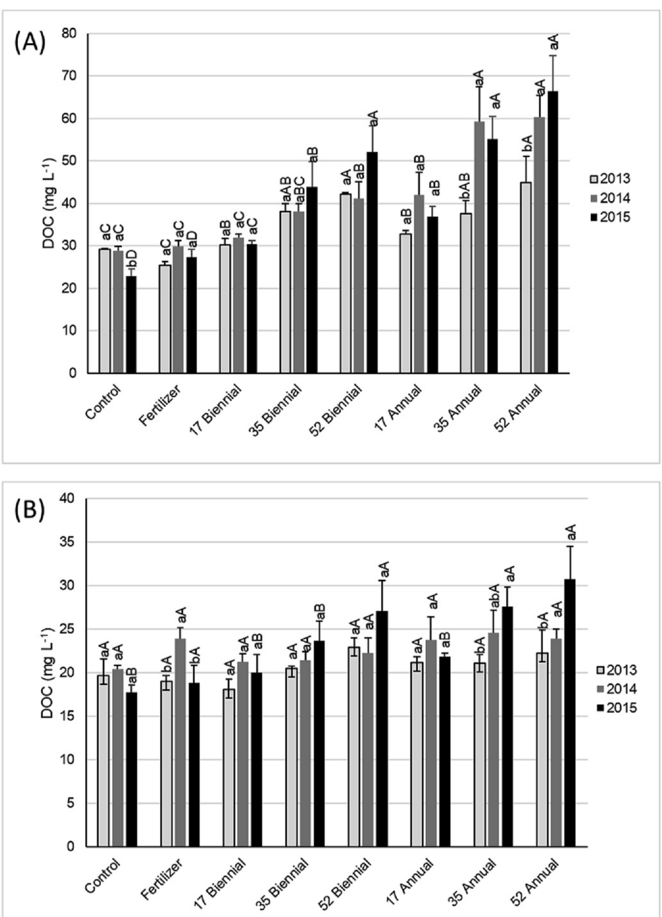

Fig. 2. DOC content for the soil extracts in 0–30 cm (A), and 30–60 cm layers (B) in 2013, 2014, and 2015. Numbers preceding the x-axis labels indicate application rate in t ha^{-1}. Different lower case represents significant difference within treatment across tine, and different capital letters represent difference between treatment for the specified year ($p < 0.05$). Error bars indicate standard error of mean.

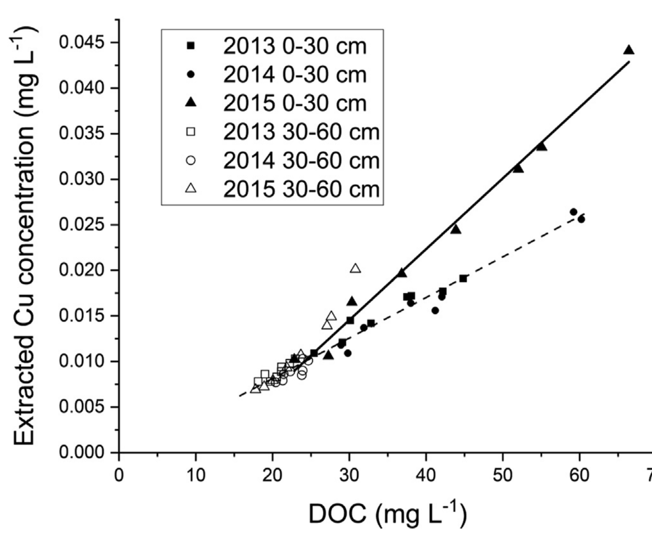

Fig. 3. Water extractable Cu concentrations vs DOC concentrations in soil extracts for all amendment rates, years and depths. The lines are drawn indicate linearity ($R^2 > 0.95$) of two clusters of data (2015 0–30 cm; and 2013, 2014 0–30 cm).

dissolved P concentrations, and DRP in the water extracts (Fig. 4 and Supplementary Material Figure S3 and Table S6). These correlations become slightly stronger with time, probably because total loading of P and Cu increased with continued manure application. Phosphate and DOC are both negatively charged molecules, and thus adsorption can be competitive. Zhang and Zhang (2010) evaluated the effect of P application on DOC and Cu, Cd, and Zn leaching in soils and showed that at high P application rates (500 mg kg^{-1} P), DOC leaching increased by 145%. The effect of P on DOC and metals leaching was greater in sandy soils than in loamy soils (Zhang and Zhang, 2010). It is likely that sorption of P increases the negative charge on the soil surface, making it more difficult for sorption of DOC, thus making DOC more mobile and increasing the mobility of DOC-complexed metals.

Extracted P concentration and DOC concentration were highly correlated; by the third year of application, Pearson's correlation coefficient was near one (Fig. 4 and Supplementary Material Table S6). The correlation between extracted P concentrations and E_2/E_3 trended more negative with time, and the association with $SUVA_{254}$ became more positive with time. While it is expected that adding manure increases extractable P and DOC, the increase in correlation between molecular properties of DOC, and extractable DOC and P concentrations indicate that over time, the interactions are increasing. Working with a loam soil from agricultural land, Sharma et al. (2017) observed that soluble organic carbon increased with P application rates, and that the soluble forms of both elements were linearly correlated ($R^2 = 0.79$). Increased correlation between P and DOC over time suggest that phosphate is associated with the organic compounds, which would increase P mobility through the soil profile. In addition to affecting DOC adsorption to soil particles, available P can facilitate soil organic matter degradation by stimulating microbial activity (Mao et al., 2017), which could either increase or decrease the concentration of DOC depending on the degree of mineralization and production of byproducts. Song et al. (2011) observed that after one year of amendment, P application stimulated microbial growth and consequently, increased DOC concentration in soil pore water.

Copper complexation by DOC increases as pH increases due to the high affinity of DOC ligand functional groups for Cu (Lu and Allen, 2002). There was no significant difference in soil pH with treatment: average soil pH amongst all treatments was 7.8 ± 0.023 (95% confidence interval). In soils with pH greater than ~6, such as the ones used in this study, functional groups on the DOC molecules are mostly deprotonated, resulting in decreased proton competition and greater affinity of DOC to bind metals (Lu and Allen, 2002; Chen et al., 2013).

Extractable Cu and P correlations with Fe increased in each subsequent year (Fig. 4 and Supplementary Material Table S6). Correlation coefficients between Fe and DOC also increased after each year's manure amendment. Given the very low solubility of Fe at alkaline pH, the dissolved Fe (filtered through 0.45 μm membrane) must be associated with DOC as complexed ions or colloids. The Cu can be associated either directly to the DOC via complexation, or can adsorb on Fe mobilized by the DOC. The increasing P—Fe correlation is typical for P solubility in soils and is caused by association of P with Fe that is complexed with DOC as a ternary complex.

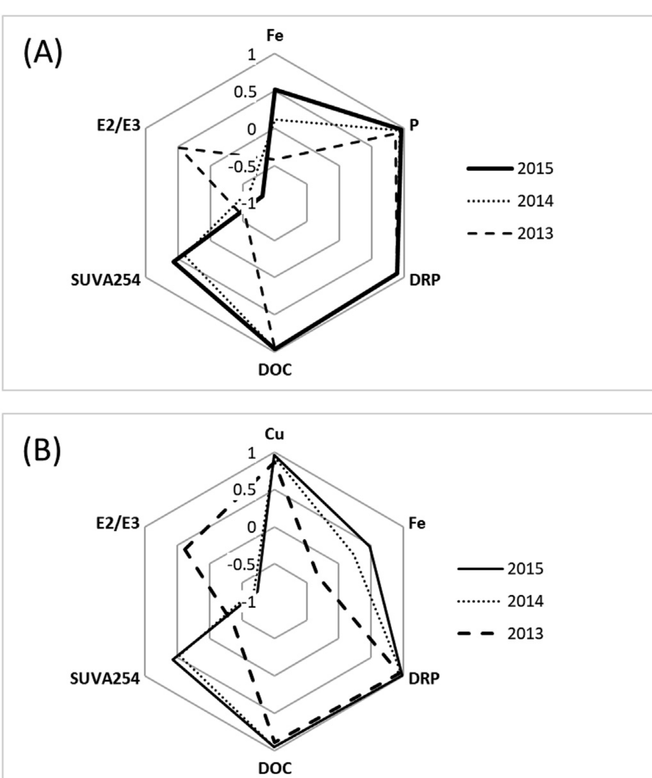

Fig. 4. Pearson correlations observed for water extractable Cu (panel A) and total dissolved P (panel B) in 2013, 2014 and 2015, 0–30 cm soil depth.

3.4. UV/VIS spectroscopic characterization of DOC

The absorbance of DOC at a specific UV/Vis wavelength ($SUVA_{254}$) is used to study molecular characteristics, including the degree of aromaticity (Corvasce et al., 2006; He et al., 2016; Kikuchi et al., 2017). The correlation between Cu and the aromaticity index ($SUVA_{254}$) became more positive after each successive annual application (Fig. 4). DOC was also analyzed using FTIR spectroscopy and showed dramatic changes in molecular structure with increased manure loading rate (Supplementary Material Figure S2), thus supporting the UV/Vis conclusions.

The $SUVA_{254}$ of extracted DOC increased in the surface with time (Fig. 5), but decreased with soil depth (Supplementary Material Table S5). This behavior suggests that either preferential flow of structurally simple molecules (i.e., less aromaticity) to deeper soil horizons is occuring, or retention of more complex and aromatic DOC molecules by mineral components of deeper soil horizons prevents them from being extracted with water (Corvasce et al.,

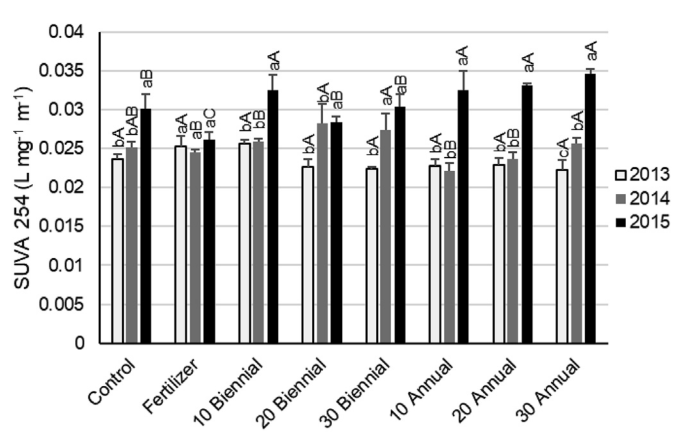

Fig. 5. $SUVA_{254}$ values for the soil extracts in 0–30 cm in 2013, 2014, and 2015. Different lower case represents significant difference within treatment, and different capital letters represent difference between treatment ($p < 0.05$). Error bars are standard error of mean.

2006). Hassouna et al. (2010) proposed that several seasons may be needed to observe significant changes in $SUVA_{254}$ in soils with manure application. However, significant differences in the $SUVA_{254}$ values between the surface and subsurface soils from the manure-amended soils used in this study were observed within 3 years (Fig. 5 and Supplementary Material Table S5), providing evidence that high manure application rates accelerate enrichment of distinct organic matter compounds in the subsurface.

The ratio of the peak intensity E_2/E_3 is used to track changes in the relative size of organic matter molecules (Thomsen et al., 2002; Mouloubou et al., 2016), and is an independent measure of the aromaticity ($SUVA_{254}$) as estimated by molecular weight. The negative correlation of extractable Cu concentrations with E_2/E_3 became stronger with time (Fig. 4), suggesting that DOC associated with extractable Cu has more aromatic components and larger molecular weights. Chen et al. (2013) found that high molecular weight (HMW) DOC from riverine samples generally showed stronger binding affinities to Cu^{2+} and Hg^{2+} than low molecular weight (LMW) DOC. Chen et al. (2013) proposed that the HMW DOC has structural (e.g., aromatic functional groups) or compositional advantages such as a diversity of functional groups that favor the formation of strong metal binding.

The degree of DOC aromaticity has been related to increases in complexation of Cd, Ni, and Zn (Baken et al., 2011; Cornu et al., 2011) and rare earth elements (Gangloff et al., 2014). Amery et al. (2010) showed that Cu affinity for DOC at low Cu^{2+} activity in wastewater, soil, pig manure, and sewage sludge was linked to aromaticity as determined by $SUVA_{254}$. Thus, increased aromatic DOC components occurring in the dairy manure-amended soils appear to facilitate Cu bonding and promote Cu transport through the soil profile in the form of a Cu-DOC complex. The increasing correlation between extractable Cu and $SUVA_{254}$ (Fig. 4) suggests that the relatively high water extractable Cu content in subsurface soils (Fig. 1) is due to increased mobilization of Cu bound to high molecular weight DOC.

The association of Cu with DOC compounds depends on the hydrophobic/hydrophilic nature of the DOC (Hur and Lee, 2011). Chen et al. (2015) used two-dimensional correlation spectroscopy and synchronous fluorescence and infrared absorption spectroscopy to explore the binding process of Cu and humic acid (HA) and concluded that the Cu binding to HA involved functional groups with an affinity as follows: carboxyl > C–O of polysaccharides > phenolic groups > aryl > amide > aliphatic groups. Phenolic and aromatic carboxylic groups may form highly stable ring structures with Cu, with a high stability constant (Manceau and Matynia, 2010; Chen et al., 2015). Fuentes et al. (2013) studied Cu binding by several humic acid materials and proposed that Cu ions selectively complex to phenolic functional groups and O-alkyl functional groups on aromatic molecules. Wen et al. (2014) studied the effect of short- (3 years) and long- (22 years) term fertilization (NPK and NPK + swine manure) on Al-DOC bonds with soils and observed that fertilization changed the Al-binding characteristics, as well as the aliphatic groups within the DOC. Future research should use advanced molecular analysis to gain additional details of the variances in DOC in the different soil depths so that knowledge of how these different forms of DOC impact Cu mobility in manure-amended soils.

4. Conclusions

After 3 years of dairy manure application, water extractable Cu concentration was greater in the surface and subsurface layers of manure-amended soils as compared to non-amended control soils and traditional mineral fertilizer-amended soils. The extractable Cu concentration was greatest in the plots that had the highest manure amendment rates (35 t ha^{-1} and 52 t ha^{-1}, dry weight). The UV/Vis parameters $SUVA_{254}$ and E_2/E_3 became increasingly more correlated with Cu concentration in the extracts, suggesting that DOC is becoming more enriched with high molecular weight aromatic compounds that have greater Cu complexation than low molecular weight organic compounds, which will enhance Cu mobilization. The 10-fold increase in extractable Cu concentration after only 3 years of manure application shows the risks of amending dairy manure with elevated concentrations of Cu to soils; especially at high amendment rates.

Results from this study show that DOC is an important factor in Cu mobility in dairy-manure amended alkaline soils. Once highly stable Cu-DOC complexes form, Cu will have increased mobility and greater potential for transport deeper in the soil profile. Because of enhanced Cu-mobility, soil health and water quality may be negatively impacted by manure application to soils. Thus, monitoring is required in manure-amended soils to ensure that Cu concentrations do not exceed a critical threshold. While it is unclear what the threshold is, this research shows that dairy manure amendment to soils will increase Cu availability and provides information about the processes controlling Cu mobility and availability, which can be used to develop best management practices. To prevent Cu loading to soils, the best solution for future dairy waste management is better management of Cu in animal production to reduce Cu concentrations in manure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.12.070.

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