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Impacts of water treatment residuals on soil ecology.

Assessing the impacts of land spreading water treatment residuals on the anecic earthworm *Lumbricus terrestris*, soil microbial activity and porewater chemistry

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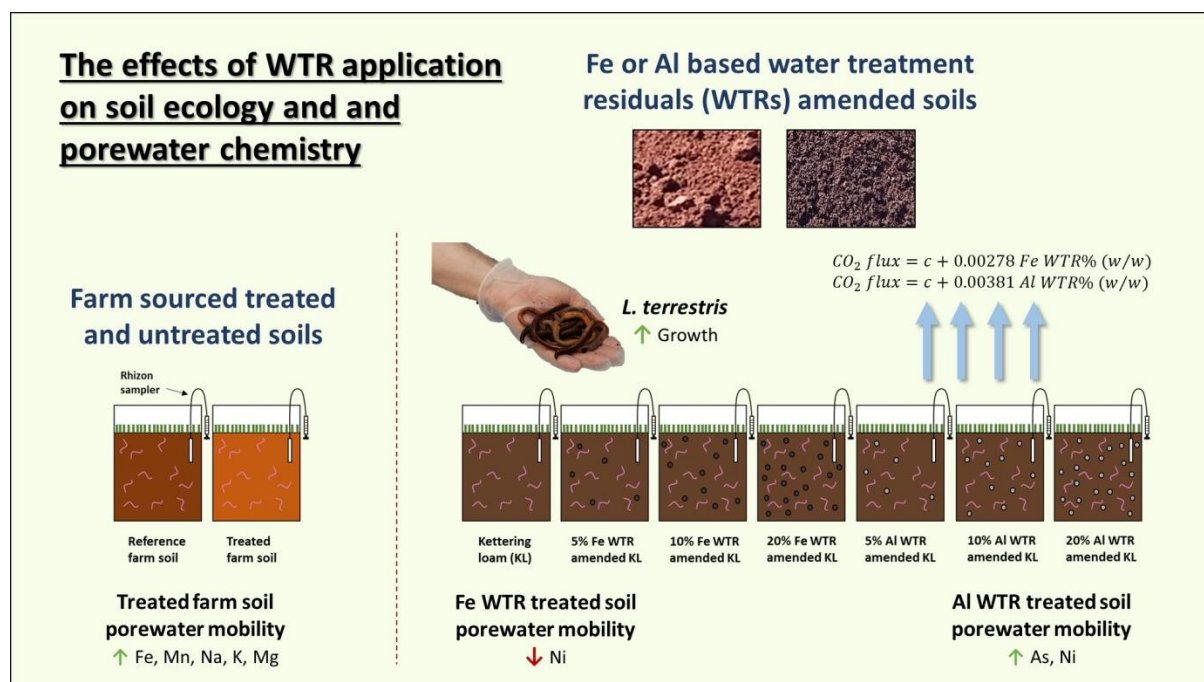
Abstract: Water treatment residuals (WTRs), by-products of drinking water clarification, are increasingly recycled to land to promote circular economy and reduce disposal costs, yet there is a lack of published literature on their effects on soil ecology. In the present study, the effects of WTRs on earthworm growth, soil respiration, and soil porewater chemistry are investigated throughout a seven-week outdoor mesocosm trial. WTRs derived from both aluminium and iron coagulants

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were applied to a loam soil at 0-20 % (w/w). Additionally, soil from a field that had received long-term WTR applications and that of an adjacent non-treated reference field were included in the study. Earthworm mass increase was significantly higher in all but one laboratory treated soils when compared to the control. Furthermore, a linear regression model can be used to predict increases in weekly soil respiration based on the application rates of both Al and Fe WTRs. In addition, a significant increase in soil respiration was observed from the treated farm soils during the first four weeks of the trial. Measured sodium, magnesium, potassium and iron porewater concentrations were higher in the treated farm soils than reference site soil in a majority of samples, although these differences may be related to land management. Laboratory treated soils had elevated porewater arsenic concentrations (e.g. $\sim 17 \mu\text{g L}^{-1}$ in controls vs $\sim 62 \mu\text{g L}^{-1}$ in the 20 % w/w Al WTR treatment in week 1), while porewater nickel concentrations were respectively elevated and lowered in Al WTR and Fe WTR amended samples. Overall, observed disturbances to soil ecology were determined to be minimal.

Graphical abstract



Keywords: Earthworms, Pore water, Soil ecotoxicology, Water treatment residual, Soil respiration

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1. INTRODUCTION

Water treatment residuals (WTRs) are a by-product generated during treatment of drinking waters. Their main component is determined by the flocculants used in the treatment process, the most common of which are Alum (aluminium sulphate), iron chloride and iron sulphate, leading to WTRs being referred to as Al WTRs or Fe WTRs respectively (Turner et al. 2019). Typically, drinking water purification

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produces around 10-30 mL of WTRs for every litre of water clarified (Dassanayake et al. 2015) and, while current global production figures of WTRs are difficult to obtain, older estimates suggest that 10000 t d⁻¹ are produced globally (Waite and Dharmappa 1993). WTRs are regularly applied to land, which is considered to have environmental benefits, including a liming effect, adding organic matter, and the immobilisation of a variety of contaminants and excess nutrients such as Cu, Ni, As, Cd, Pb, Zn and P (Elkhatib and Moharem 2015; Garau et al. 2014; Nagar et al. 2015). However, while a number of studies have explored aspects of potential chemical impact of WTRs on the environment, very few have specifically explored the ecological impacts.

Earthworms are one of the most abundant terrestrial invertebrates in the temperate regions and important 'ecosystem engineers'. They are well suited to use in monitoring potential contamination or other soil impacts, due to the constant contact between their permeable skin and the surrounding soil which makes them sensitive to changes in the chemical and physical soil properties (Paoletti et al. 1998; Roubalová et al. 2015). Indeed, Spurgeon and Hopkin (1999) demonstrated that earthworm abundance and biomass decreased with proximity to a Pb/Zn/Cd smelting works in the UK. However, little research has been done to date on the effects on earthworms following soil amendment with WTRs, although an initial short-term (14 day) study by Howells et al. (2018) found that earthworms exposed to 0-20 % w/w WTR amended soils did not have their biomass, survival or reproduction affected. However, the same study found that earthworms avoided soils amended to ≥ 10 % w/w of Fe WTR and to 20 % w/w Al WTRs which, together with a lack of published data relating to potential ecological impacts warrants further investigation.

The influence of land application of WTRs on soil microbial activity is also yet to be fully understood. It is known that WTRs themselves are a source microorganisms

such a *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, and *Planctomycetes* (Oliver et al. 2011; Würzer et al. 1995; Xu et al. 2018), but the overall effect of WTRs addition is still not certain. Pecku et al. (2006) found that an application of 300 t ha⁻¹ of WTRs had no detrimental effect on soil respiration or microbial diversity. However, mixed results were obtained by Garau et al. (2014) when applying Fe WTR amendments (3% w/w addition rate), with an increase in the amount of culturable heterotrophic bacteria and actinomycetes and a decrease in the amount of heterotrophic fungi. They concluded that the overall microbial biomass of samples remained approximately constant although the suite of species present changed. A commonly employed method of estimating overall microbial activity is the measurement of soil CO₂ efflux. In soils, carbon dioxide is primarily released through microbial decomposition of soil organic matter (SOM), while a few percent is caused by root interactions (e.g. root respiration and rhizo-microbial respiration) and chemical oxidation of organic matter (Kuzyakov 2006; Raich and Schlesinger 1992; Smith et al. 2008). Therefore, monitoring CO₂ efflux can reveal changes in microbial activity following amendments and other treatments.

The potential for WTRs to leach constituent elements, particularly Al, into the surrounding environment is often considered to be the greatest concern in relation to land application of WTRs. In some countries this is accounted for in legislation, for example in England and Wales the application of Al WTRs and Fe WTRs are limited to soils above a pH of 6.0 and 5.0 respectively, due to the increased mobility of Al and Fe in soils at low pH (Environment Agency 2013). The importance of these restrictions was highlighted by Howells et al. (2018), who found that the amount of leachable (0.001 M CaCl₂) Al from the Al WTRs increased from 4.5 mg kg⁻¹ to 382 mg kg⁻¹ when decreasing the pH from 5.5 to 4.4.

The present study aims to confirm that addition of WTRs to soil does not alter the porewater chemistry (in terms of metal and metalloid concentrations) to any substantial degree that would inhibit plant or soil biota health, and that no negative effects on earthworm growth occurs. Furthermore, we aimed to test for the first time how WTRs addition affected overall microbial activity measured by microbial respiration

2. MATERIALS AND METHODS

2.1. Soils and Water Treatment Residuals

Soil treatments were prepared in the laboratory by adding Al WTRs or Fe WTRs to a commercially supplied natural soil, Kettering Loam (Kettering, Northamptonshire, UK, supplied by Boughton Ltd, www.boughton.co.uk). This soil was steam sterilised before purchase (~4 years prior to use) and then stored under cover outdoors.

Kettering loam was selected because it has been previously used in earthworm studies and is known to be suitable for a range of soil dwelling species (Brami et al. 2017; Butt 2002; Rajapaksha et al. 2014). In addition, sandy clay soils were collected from two adjacent agricultural fields in South Wales to compare the impacts of long-term WTR application in a parallel study. One field had received heavy applications of WTR solids for many years (most recently 135 t ha^{-1} in 2015 and 92 t ha^{-1} in 2016) while the other acted as a control and had received no (or only incidentally) applied WTRs (henceforth referred to as 'Farm treated' and 'Farm-reference' soils respectively). It is worth noting that the treated area had more commonly been used as arable land while the reference area had more commonly been used for pasture. These soils were all dried at 105° C and sieved to 4 mm in order to ensure sample homogeneity. The WTRs used in the present study were sourced from treatment

plants in the south west of the UK. The Fe WTRs came from the same water treatment plant as the WTRs applied to the treated farm soil. As per the soils, the WTRs were dried at 105 °C and crushed using a jaw crusher before being sieved (to 2 mm).

The water content of the WTRs, as received, were 25.85 % and 65.27 % (w/w) for the Al and Fe WTRs respectively. Soils and WTRs were characterised for pH by 1:5 solid: deionised water suspensions, organic matter by loss on ignition at 550°C, and water holding capacity (WHC) by saturation and drainage (Table 1).

2.2. Mesocosm setup

Experiments were conducted in semi field conditions using purpose built outdoor mesocosms. These consisted of cylindrical pots with a depth of 12.5 cm and a diameter of 14.5 cm. Drainage holes were drilled in the base of the pots. Velcro was attached to the inner rims of the pots in order to discourage earthworm escape. The Kettering loam soil was hand mixed with the Al or Fe WTRs at rates of 0 %, 5 %, 10 % and 20 % by dry weight. The 5 % WTR application rate was selected as an upper level of what is ever likely to be used in land spreading practices on agricultural soil (i.e. 5 % equates to $\sim 120 \text{ t ha}^{-1}$, assuming a soil density of 1.2 g cm^{-3} and depth of 20 cm), whereas the 10% and 20 % application rates were selected as extremes to determine the extent of application required to bring about ecological effects. For reference, within England and Wales, WTR application is limited to 250 t ha^{-1} per annum, although this is often further reduced for WTRs with high solids content. Each mesocosm was filled with 1.5 kg of the corresponding substrate. Farm and laboratory soils were wetted to 60 % and 50 % WHC respectively. Once installed outdoors the water content was allowed to fluctuate naturally and was checked weekly to ensure they did not dry out.

Ten sexually mature (visible clitellum) *Lumbricus terrestris* earthworms were rinsed with deionised water, patted dry and weighed, and then placed in each pot (earthworms were originally sourced from Yorkshire Worms, Goole, UK). On average, there was 24.0 g of earthworms per kg of substrate in each mesocosm. This density of earthworms is lower than the 50-60 g of soil per earthworm specified in ISO and OECD protocols for earthworm studies, but is in keeping with rates recommended by others for long term tests (e.g. Bart et al. (2018)). *L. terrestris* is a species of earthworm that falls within the anecic ecological subgroup. Anecic earthworms characteristically create and live within permanent vertical burrows. This species was chosen because they are commonly found in mineral soils, unlike other species often employed in ecotoxicology assays such as *Eisenia fetida* and *Eisenia andrei* that generally live in high organic matter substrates such as composts and litters. Rhizon samplers (Rhizosphere Research Products, The Netherlands) were installed at 5 cm depth in all mesocosms. Once prepared, mesocosms were sown with 3 g of ryegrass (*Lolium Perenne*) seeds to create an environment that reflected a pasture soil scenario and would act as a food source for the earthworms. The mesocosm treatments thus included two farm soils, six laboratory amended soils and one control soil (non-amended Kettering Loam) (figure 1). Four replicates (n=4) were prepared for each, resulting in 36 mesocosms being assembled in total. For the duration of the study the mesocosms were situated in an enclosed (fenced off) outdoor site. Mesocosms were elevated off the ground on wooden frames with plastic mesh around them to prevent access to birds and other wildlife but otherwise keep conditions consistent with field conditions (i.e. natural field temperatures and rain conditions for central UK during October-November 2018) (figure 2).

2.3. Earthworm, porewater and CO₂ flux measurements

Earthworms were recovered from mesocosms at the end of the experiment (after 49 days) via hand sorting. They were washed and weighed in the field to measure their average weight for comparison to weights before the experiment. The change in average weight of earthworms was chosen as an indicator of earthworm health.

Porewater samples were collected weekly over a five-week period via the installed rhizon samplers. These samplers comprised of a porous ceramic-like filter attached to a PVC tube through which water can be extracted using a syringe under vacuum conditions. Collected samples were acidified with analytical grade HNO₃ and analysed via ICP-MS (Agilent 7500ce) along with certified solution standards. Soil porewater sampling has many benefits compared to other measures of element bioavailability (such as extraction with neutral salt solutions), because it directly samples the solution that plant roots and soil invertebrates experience and it does not rely on an artificial reagent to displace solutes. Moreover, the rhizon sampler method allowed repeated samples to be taken in a non-destructive manner.

CO₂ flux was measured weekly over a six-week period using a PP Systems- EGM-5 Portable CO₂ analyser. This method works by placing the device's chamber (surface area of 78.5 cm²) on the soil surface to produce an airtight seal, then air is pumped through the chamber and the difference between CO₂ concentrations in the inflowing and outflowing air streams determines the CO₂ flux from the soil. The CO₂ flux measurements were conducted over a 60-second period for each sample after a 15-second purge time and 12-second equilibration time.

2.4. Additional data sources and statistical methods

Elemental analysis of WTRs were also determined by a certified, commercial laboratory via USEPA Method 3050B, following standard QA/QC protocols (see supplementary material table 1). In summary, 0.5g of dried material was digested in 12 ml of aqua-regia (9 ml HCl + 3 ml HNO₃) in a hot-block digestion set at 125 °C. The digestate was then diluted to 50 ml with DI water and elemental concentrations determined by either ICP-OES or ICP-MS depending upon the concentration present. Meteorological data was collected from a weather station situated ~ 500 m from the site of the experiment, allowing highly accurate hourly weather data to be obtained. All data was processed, analysed and statistically assessed using Microsoft Excel and Minitab, employing linear regressions, T-tests and ANOVAs following appropriate checks for adherence to normality and associated underlying assumptions.

3. RESULTS

3.1. Earthworm weight change

When assessed with a standard ANOVA approach there were no significant differences found between the weight changes of earthworms in treatments and controls for any of the farm treated or laboratory amended soils (Al WTR treatments vs control ANOVA $p = 0.064$; Fe WTR treatments vs control ANOVA $p = 0.095$; farm treated vs reference soil t-test $p = 0.264$), however, prompted by apparent visual trends (figure 3), assessment via one-sample t-tests revealed significantly higher earthworm mass increases in all of the Al-WTR treated soils and the 5% and 20% Fe-WTR treated soils compared with the control ($p < 0.05$), but for the treated farm soils there was still no detectable significant differences from the reference soil ($p = 0.20$). A statistically significant positive relationship was also identified by linear regression analysis between Fe WTR addition (% w/w) in laboratory amended soils and

earthworm mass increase, although only a low proportion of the variance could be accounted for by this model (R^2 0.30, $p = 0.027$).

3.2. CO_2 flux (Soil respiration)

Field treated farm soils. There was a significant difference between CO_2 fluxes of the treated and reference farm soil mesocosms in weeks 1 to 4, but not weeks 5 and 6, with the treated farm soils having a higher CO_2 efflux in every case (t-tests $p < 0.05$, figure 4).

Laboratory amended soils (Kettering Loam). When examined on a weekly basis, the only significant difference in CO_2 flux observed following laboratory additions of Al or Fe WTRs to Kettering loam was recorded during week 6 under Fe WTR application (ANOVA $p > 0.05$ in every other case, figure 4). Multiple linear regression analysis of the overall data set indicated that the main predictor of gas flux was air temperature ($p < 0.001$, R-sq 0.60). However, when considering the whole data set on an independent weekly basis (allowing a degree of normalisation for air temperature), regression results indicated that the application rate of Al and Fe WTRs could be used to predict for the CO_2 flux of soils ($p = 0.004$ and 0.018 respectively), and accounted for a large amount of the variance ($R^2 = 0.7405$ and 0.7782 respectively). Regression equations for Al and Fe WTR amended CO_2 flux can be seen in equations 1 and 2 respectively.

$$CO_2 \text{ flux} = c + 0.00381A \text{ \#Eq. 1}$$

$$CO_2 \text{ flux} = c + 0.00278A \text{ \#Eq. 2}$$

Where c is a constant that varies from week to week and A is the application rate expressed as a dry weight percentage.

3.3. Pore waters.

Farm soils (field treated and reference). Porewater element concentrations were assessed week by week and evaluated for differences between the field treated and reference farm soils via t-test comparisons. Interestingly the Al concentrations of porewaters were not significantly different. Concentrations of Cu, Zn, As and Pb only differed significantly (treated vs reference soil) during one week over the entire sampling period, however these were all lower in the treated soils, aside from Pb which was marginally enriched in treated soils ($0.3 \mu\text{g L}^{-1}$ vs $0.07 \mu\text{g L}^{-1}$ in treated vs reference farm soils during week 3) (Supplementary material table 2). More notably, Na, Mg, K, Mn and Fe concentrations differed significantly during at least three weeks of the study (figure 5). In every case, these five elements were elevated in treated soils, for example, Fe concentrations were $339 \mu\text{g L}^{-1}$ and $8062 \mu\text{g L}^{-1}$ during week four in reference and treated farm soils respectively.

Laboratory amended soils (Kettering Loam). The Al concentrations in porewaters of Al- or Fe-WTR laboratory amended soil did not differ significantly from controls (Figure 6), neither did the concentrations of Fe except for under Al-WTR application in week 5 (Figure 6). However, additions of Al WTRs significantly elevated the As concentration compared with the untreated control in every week except week 3 (Figure 7), with the increase typically being at least 3-fold at the highest application rate (e.g. from $\sim 17 \mu\text{g L}^{-1}$ in the control to $\sim 62 \mu\text{g L}^{-1}$ in the 20 % Al WTR treatment in week 1). The addition of Fe WTRs also increased porewater As concentration in three of the weeks during which porewaters were monitored, but the increases were more modest than in the case of Al WTRs (Figure 7). Additionally, in the case of Fe WTR treated soils, Ni concentrations in porewaters were elevated during the same weeks As enrichment was observed, by a factor of 1.3 – 2.0 (Figure 7). Contrastingly,

Al WTR treated mesocosms had reduced Ni concentrations in every week of sampling. Other elements, particularly Cr, were also either decreased in concentration or were unaffected by Al and Fe WTRs addition. The large increases in Fe concentrations observed in the amended farm soils were not replicated in Kettering loam amended with Fe-WTRs in the laboratory.

4. DISCUSSION

Earthworm mass increase was found to be higher in the laboratory-amended soils, which contrasts with results from laboratory experiment reported by Howells et al. (2018) who applied WTRs at similar application rates (0, 5, 10 and 20 % by weight), and found that there was no significant difference in the growth rate of a different earthworm species (*Eisenia fetida*). Between the present study and that of Howells et al. (2018) two of the three principal earthworm subgroups (anecic and epigeic) are covered and therefore, there can be a degree of confidence that WTRs application is unlikely to have any negative impacts on earthworm growth when they are applied at typical rates. The positive relationships between WTR application rate and earthworm weight increase found in the present study may be due to the organic matter additions from WTRs. The fact that the farm treated soils showed no difference in earthworm growth might indicate that any enhancement generated by WTRs addition has a time-limited effect.

The subtler differences between laboratory amended and non-amended Kettering loam gas fluxes when compared with those between the treated and reference farm soils could indicate that previous soil conditions and management practises of the farm soils may also have played a role in the differences in flux observed or that the pre-treatment of the Kettering loam including steam sterilisation ~4 years prior to use may have influenced the microbial response observed during the experiment. The

surface WTR amendments may have also led to indirect effects on CO₂ flux due to changes in water holding capacity and bulk density and albedo. However, regression analysis in the present study did indicate that Fe and Al WTRs could influence soil respiration at higher application rates. These results differs from those reported by Mukherjee and co-workers (2014a; 2014b), who found no effect on CO₂ emissions in soils amended with low rates of WTRs (0.5% w/w), but is consistent with those from Pecku et al. (2006) who observed a general increase in CO₂ flux after higher applications rates of WTRs (up to 25% w/w) when measured in 24 h jar incubation experiments. It is possible that the increase in organic matter and/or alteration or stimulation in the microbial community introduced by the addition of WTRs may contribute to differences in gas flux (and therefore microbial activity) over longer periods or at higher application rates, and therefore understanding the underlying mechanisms (including changes to microbial species suites) is an avenue for further research.

Difference in the porewater Al and Fe concentrations of laboratory-amended and non-amended Kettering loam soils were rarely statistically significant (the only exception is Fe in week 5; figure 7H), suggesting both Al and Fe from the WTRs are non-leachable under these conditions. This is likely, in part, due to the pH of the soils which were all above 5.5 (i.e. above the point where Al and Fe become more readily mobile). The As concentration, on the other hand, was notably higher in the WTR amended Kettering loam soils (in the region of ~13 – 70 µg L⁻¹ at all application rates). The As concentrations in the present study were below typical toxicity thresholds reported for porewater As, however the highest level observed was comparable to the 50 % effect concentration for cucumber plants recorded in one sensitive soil from Australia (viz. 60 µg L⁻¹; Kader et al. (2017)). Certain regulations

in some jurisdictions require WTRs to be periodically analysed for elemental content if they are to be applied to land, and such analysis can be used to set limits on how much can be applied. This is the case in the UK, where the WTRs used in the present study were obtained, and previous unpublished analysis of Al WTRs from the same water treatment plant identified that As is an element that can limit the amounts of the material that can be applied to land under those regulations. The porewater results of the present study indicate that such a limit is a prudent precaution because they demonstrate that As in these WTRs is potentially mobile to some degree. However, regional differences in WTR composition and properties must be considered, as past studies have produced mixed results. For example, a study by Chiang et al. (2012) found that during sorption/desorption tests of goethite and WTR mixtures the leaching of As was proportional to the WTR content of the blend (i.e. WTRs contributed leachable As). However, Al WTRs from elsewhere have previously been shown to significantly reduce As mobility in treated soils (e.g. Garau et al. (2014); Silvetti et al. (2014)). Neither the modest release of Ni by Fe WTRs nor its sorption by Al WTRs, as observed here for the laboratory amended soils, have been previously documented in the literature. In contrast to the Kettering loam laboratory amended soils, the field treated farm soils showed no changes in porewater Ni concentrations. Indeed, there were few examples of similar trends in porewater chemistry between the laboratory treated Fe WTR-Kettering loam samples and the treated farm samples. It is possible that differences in the rates of WTR application and previous soil management at the farm, in addition to any aging effects and mineralogical composition, could have given rise to the dissimilar porewater results. Aging effects have been reported by Agyin-Birikorang and O'Connor (2009), who found that Al mobility decreased in WTRs over time, and a similar process could potentially also occur for other

elements. However, to our knowledge, no further investigation has been done on the subject. Reductions in other porewater elemental concentrations, particularly Cu in Al WTR amended samples and Cr in all of the amended samples, can be accounted for by the high sorption capacity of WTRs. For example, Zhou and Haynes (2011) established that, even at a low pH of 5, Al WTRs added to test solutions (10 g L^{-1}) could sorb up to 114 g kg^{-1} Cr and up to 52 g kg^{-1} Pb at the highest metal doses imposed. Similarly, Ngatenah et al. (2010) found that 100% of the Cu in a 65 mg L^{-1} Cu solution was removed using 2 g L^{-1} of ground WTRs. Meanwhile, Soleimanifar et al. (2016) found that 81 % of a $100 \text{ } \mu\text{g L}^{-1}$ dose of Cu was sorbed by WTR coated mulch ($\geq 1:3$ WTR to mulch w/w) over a period of 120 minutes. It is possible that the presence of earthworm and their degradation of organic matter may have influenced mobility of some elements (Sizmur et al. 2011).

5. CONCLUSIONS

Mean earthworm mass increase was significantly enhanced in the majority of the laboratory amended soils, while no significant differences were discernible between soils treated with WTRs previously on the farm and the non-treated farm reference soils. When examined across the whole dataset, a small positive association between fresh WTR additions was identified; investigation over a longer period may help provide a better picture of these effects.

Soil porewater Al was not appreciably affected by WTR addition in either the freshly applied or field applied and aged samples, indicating that Al leaching is not likely to be a concern with these WTRs under normal field conditions. Porewater As content was largely unaffected in the farm treated soils, but was increased in the fresh laboratory amended soils when additions far above regular agricultural practises were made (up to $\sim 70 \text{ } \mu\text{g L}^{-1}$ at the highest rate of Al WTR application) and this warrants

further research. Freshly applied Fe WTRs also appeared to be a minor source of soluble Ni, but this was not observed in the farm treated soils suggesting that this affect may reduce over time. Results indicate that the elemental mobility in freshly treated soils and in aged, treated soils may vary. Generally, the leachable amounts determined in the porewaters represent a tiny fraction of the total element contents (see supplementary table 1), indicating low mobility of elements within the WTRs. Nevertheless, longer term and/or intensive leaching studies are warranted to confirm this remains the case over time.

At the rates that WTRs are commonly applied, and considering the bounds of normal field conditions, the application of WTRs are unlikely to have a negative impact on earthworms or soil respiration. Although, there is still scope for longer-term experiments to be conducted.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability statement—Data, associated metadata, and calculation tools are available from the corresponding author (T.turner@keele.ac.uk).

Disclaimer--The authors have no conflict of interest to declare.

Author Contributions statement--Tomi Turner conducted the experiment and wrote the manuscript, Rebecca Wheeler provided expertise in the waste disposal industry, Ian Oliver conceived the experiment and assisted in editing the manuscript.

REFERENCES

- Agyin-Birikorang S, O'Connor G. 2009. Aging effects on reactivity of an aluminum-based drinking-water treatment residual as a soil amendment. *Science of the Total Environment*. 407(2):826-834.
- Bart S, Amossé J, Lowe CN, Mougin C, Péry AR, Pelosi C. 2018. *Aporrectodea caliginosa*, a relevant earthworm species for a posteriori pesticide risk assessment: Current knowledge and recommendations for culture and experimental design. *Environmental Science and Pollution Research*. 25(34):33867-33881.
- Brami C, Glover AR, Butt KR, Lowe CN. 2017. Effects of silver nanoparticles on survival, biomass change and avoidance behaviour of the endogeic earthworm *allolobophora chlorotica*. *Ecotoxicology and environmental safety*. 141:64-69.
- Butt KR. 2002. Depth of cocoon deposition by three earthworm species in mesocosms. *European Journal of Soil Biology*. 38(2):151-153.
- Chiang YW, Ghyselbrecht K, Santos RM, Martens JA, Swennen R, Cappuyns V, Meesschaert B. 2012. Adsorption of multi-heavy metals onto water treatment residuals: Sorption capacities and applications. *Chemical Engineering Journal*. 200:405-415.
- Dassanayake KB, Jayasinghe GY, Surapaneni A, Hetherington C. 2015. A review on alum sludge reuse with special reference to agricultural applications and future challenges. *Waste Management*. 38:321-335.
- Elkhatib EA, Moharem ML. 2015. Immobilization of copper, lead, and nickel in two arid soils amended with biosolids: Effect of drinking water treatment residuals. *Journal of Soils and Sediments*. 15(9):1937-1946.

- Environment Agency. 2013. How to comply with your landspreading permit. 2 ed.: Environment Agency.
- Garau G, Silveti M, Castaldi P, Mele E, Deiana P, Deiana S. 2014. Stabilising metal (loid) s in soil with iron and aluminium-based products: Microbial, biochemical and plant growth impact. *Journal of Environmental Management*. 139:146-153.
- Howells AP, Lewis SJ, Beard DB, Oliver IW. 2018. Water treatment residuals as soil amendments: Examining element extractability, soil porewater concentrations and effects on earthworm behaviour and survival. *Ecotoxicology and Environmental Safety*. 162:334-340.
- Kader M, Lamb DT, Wang L, Megharaj M, Naidu R. 2017. Zinc-arsenic interactions in soil: Solubility, toxicity and uptake. *Chemosphere*. 187:357-367.
- Kuzyakov Y. 2006. Sources of co₂ efflux from soil and review of partitioning methods. *Soil Biology and Biochemistry*. 38(3):425-448.
- Mukherjee A, Lal R, Zimmerman A. 2014a. Effects of biochar and other amendments on the physical properties and greenhouse gas emissions of an artificially degraded soil. *Science of the Total Environment*. 487:26-36.
- Mukherjee A, Lal R, Zimmerman AR. 2014b. Impacts of 1.5-year field aging on biochar, humic acid, and water treatment residual amended soil. *Soil Sci*. 179(7):333-339.
- Nagar R, Sarkar D, Punamiya P, Datta R. 2015. Drinking water treatment residual amendment lowers inorganic arsenic bioaccessibility in contaminated soils: A long-term study. *Water, Air, & Soil Pollution*. 226(11):366.
- Ngatenah S, Kutty S, Isa M. 2010. Optimization of heavy metal removal from aqueous solution using groundwater treatment plant sludge (gwtps).

- Oliver IW, Grant CD, Murray RS. 2011. Assessing effects of aerobic and anaerobic conditions on phosphorus sorption and retention capacity of water treatment residuals. *Journal of Environmental Management*. 92(3):960-966.
- Paoletti M, Sommaggio D, Favretto M, Petruzzelli G, Pezzarossa B, Barbaferi M. 1998. Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. *Applied Soil Ecology*. 10(1):137-150.
- Pecku S, Hunter C, Hughes J. 2006. The effects of water treatment residues on soil respiration and microbial community structure. *Water Science and Technology*. 54(5):215-225.
- Raich JW, Schlesinger WH. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus B*. 44(2):81-99.
- Rajapaksha N, Butt KR, Vanguelova E, Moffat A. 2014. Short rotation forestry–earthworm interactions: A field based mesocosm experiment. *Applied soil ecology*. 76:52-59.
- Roubalová R, Procházková P, Dvořák J, Škanta F, Bilej M. 2015. The role of earthworm defense mechanisms in ecotoxicity studies. *ISJ*. 12:203-213.
- Silvetti M, Castaldi P, Holm PE, Deiana S, Lombi E. 2014. Leachability, bioaccessibility and plant availability of trace elements in contaminated soils treated with industrial by-products and subjected to oxidative/reductive conditions. *Geoderma*. 214:204-212.
- Sizmur T, Tilston EL, Charnock J, Palumbo-Roe B, Watts MJ, Hodson ME. 2011. Impacts of epigeic, anecic and endogeic earthworms on metal and metalloid mobility and availability. *Journal of Environmental Monitoring*. 13(2):266-273.

- Smith P, Martino D, Cai Z, Gwary D, Janzen H, Kumar P, McCarl B, Ogle S, O'Mara F, Rice C. 2008. Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*. 363(1492):789-813.
- Soleimanifar H, Deng Y, Wu L, Sarkar D. 2016. Water treatment residual (wtr)-coated wood mulch for alleviation of toxic metals and phosphorus from polluted urban stormwater runoff. *Chemosphere*. 154:289-292.
- Spurgeon D, Hopkin S. 1999. Seasonal variation in the abundance, biomass and biodiversity of earthworms in soils contaminated with metal emissions from a primary smelting works. *Journal of Applied Ecology*. 36(1):173-183.
- Turner T, Wheeler R, Stone A, Oliver I. 2019. Potential alternative reuse pathways for water treatment residuals: Remaining barriers and questions—a review. *Water, Air, & Soil Pollution*. 230(9):227.
- Waite TO, Dharmappa HB. 1993. Optimal management of water treatment plant residuals: Technical and commercial review. Sydney, Australia.
- Würzer M, Wiedenmann A, Botzenhart K. 1995. Microbiological quality of residues from drinking water preparation. *Water Science and Technology*. 31(5-6):75.
- Xu H, Pei H, Jin Y, Ma C, Wang Y, Sun J, Li H. 2018. High-throughput sequencing reveals microbial communities in drinking water treatment sludge from six geographically distributed plants, including potentially toxic cyanobacteria and pathogens. *Science of The Total Environment*. 634:769-779.
- Zhou Y-F, Haynes RJ. 2011. Removal of pb (ii), cr (iii) and cr (vi) from aqueous solutions using alum-derived water treatment sludge. *Water, Air, & Soil Pollution*. 215(1-4):631-643.

Figure legend

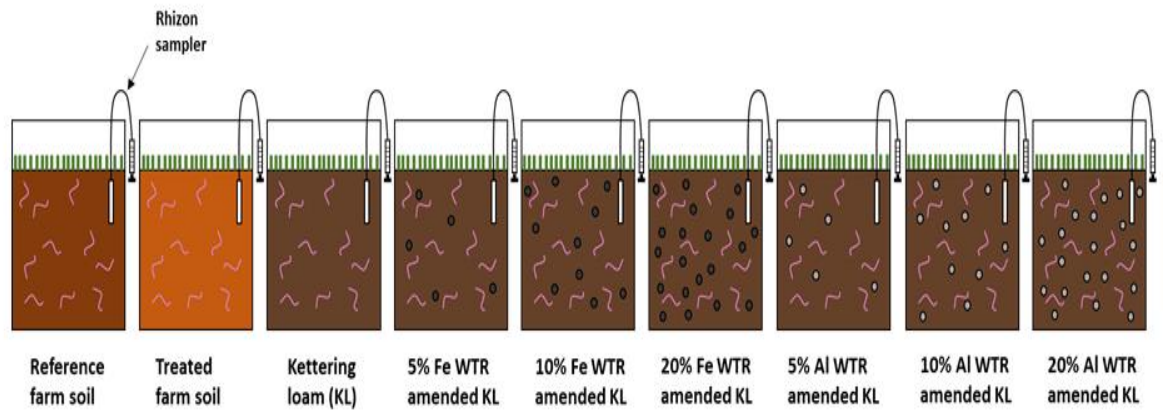


Figure 1. A summary of the different mesocosm substrates prepared for the present study.



Figure 2. A photo of the outdoor setup used for holding mesocosms during the present study.

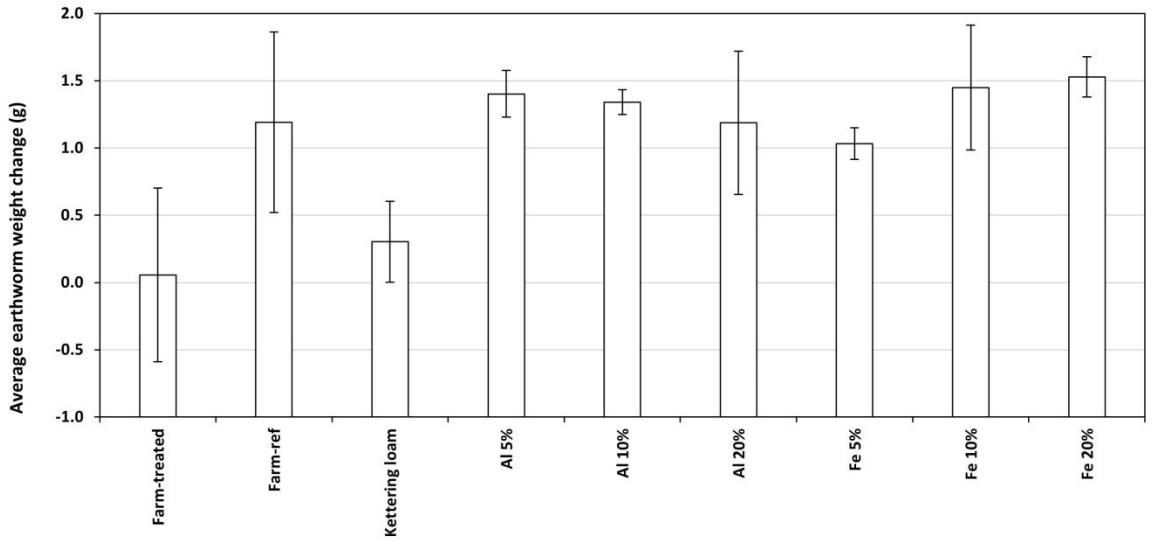


Figure 3. The average change in earthworm weight after 49 days, error bars display one standard error.

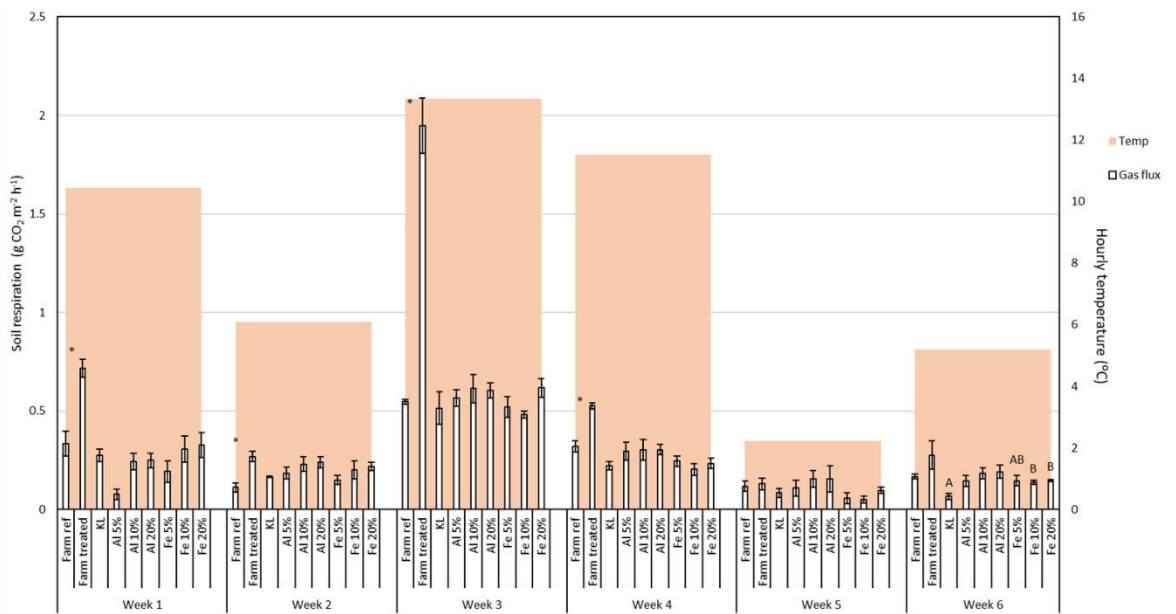


Figure 4. A summary of CO₂ flux measurements and air temperature during the six weeks of sampling; error bars display one standard error, * indicates significantly different samples, A and B indicate significant difference groupings.

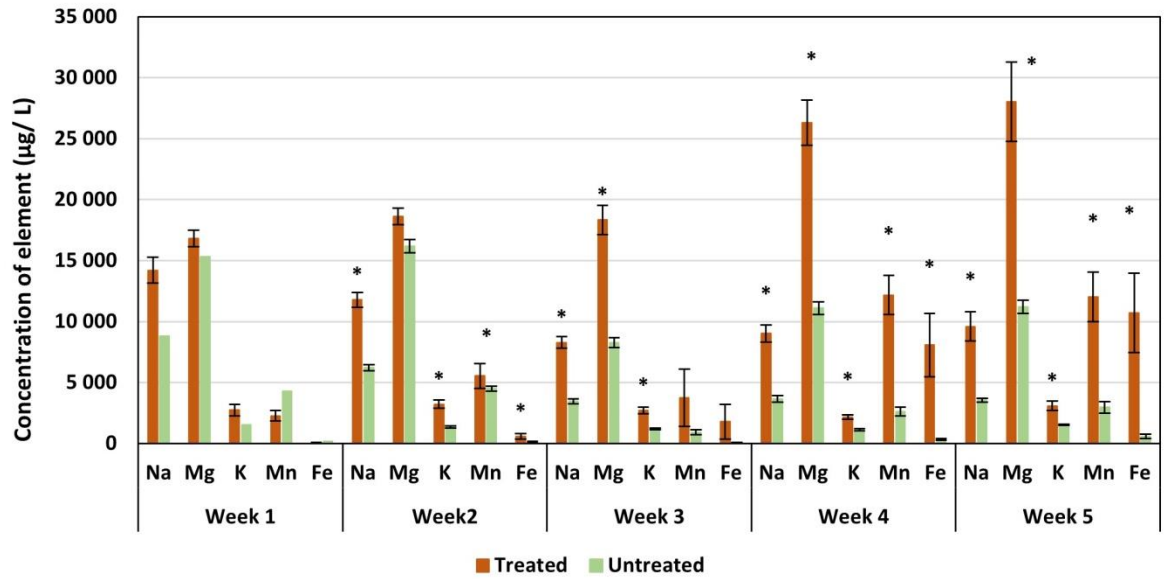


Figure 5. The concentrations of elements of interest in porewaters from farm soils, with significant differences noted by asterisks, error bars represent one standard error.

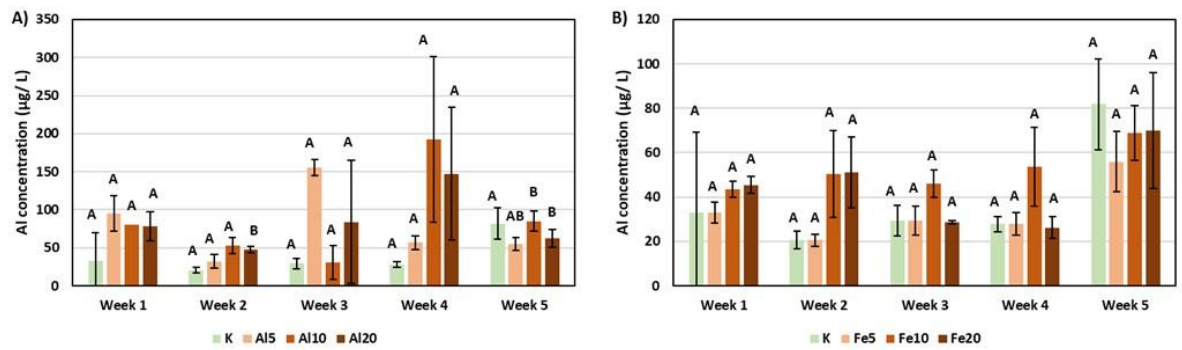


Figure 6. Mean concentration ($\mu\text{g L}^{-1}$) of Al in Al WTR (A) and Fe WTR (B) amended Kettering loam soil. Error bars display one standard error.

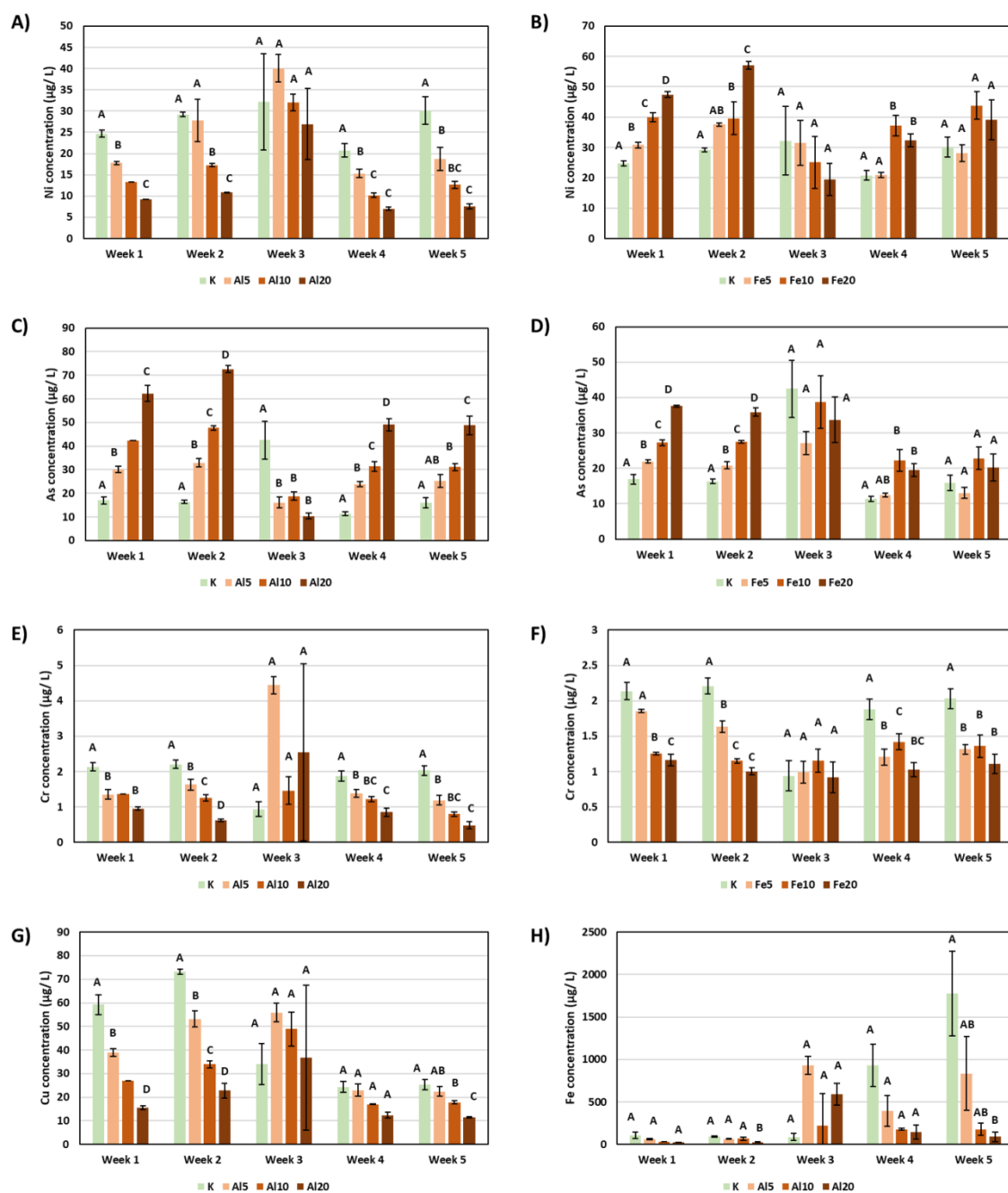


Figure 7. Mean concentration ($\mu\text{g L}^{-1}$) of nickel (A and B), arsenic (C and D), chromium (E and F), copper (G) and iron (H) in porewaters of Al WTR and Fe WTR treated Kettering loam soil. ANOVA groupings indicated by letter. Error bars display one standard error.

Table 1. Properties of the soils and water treatment residuals used in the present study.

Sample	pH	Organic content %	Water holding capacity mg g ⁻¹
Farm treated soil	7.05 ± 0.00	12.09 ± 0.07	0.76
Reference farm soil	5.79 ± 0.01	12.05 ± 0.26	0.60
Kettering loam	7.65 ± 0.02	16.05 ± 0.11	0.64
Fe WTR	7.48 ± 0.01	24.81 ± 4.56	-
Al WTR	6.2 ± 0.04	66.93 ± 0.72	-