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Classification of grasses in the Mpumalanga coalfields region and assessment of their suitability to increase the pH of acid mine drainage, for potential use in passive acid mine drainage remediation systems

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ABSTRACT

Grass samples collected from various locations in the Mpumalanga coalfields region, (South Africa), were classified and characterised using various analytical techniques to determine their physical and chemical composition. The suitability of the grasses for use in acid mine drainage (AMD) remediation was evaluated by adding a defined portion of these grasses to both synthetically prepared and real AMD collected from an AMD received dam (RD) source in the Mpumalanga coalfields region, (South Africa). This was to establish whether the grass addition was able to achieve an increase in pH, and if the effect was more notable for a particular grass type. Results showed that grass addition to AMD resulted in an increase in pH (or decrease in the hydrogen ion (H^+) concentration of synthetically prepared AMD. The addition of different grass types produced varying results, and the *Hyparrhenia hirta* (G12 and G13) and *Chrysopogon zizanioides* (G15) formally known as *Vetiveria zinanioldes* grass types produced a greater overall percentage decrease in H^+ concentration. Multiple grass additions sustained the overall high percentage decrease in H^+ concentration for the best-performing grass types and improved the overall percentage decrease in H^+ concentration of the more poorly performing *Eragrostis curvula* (G6), and *Hyparrhenia filipendula* (G5) grass types tested in synthetic AMD. The notable changes observed in cation and anion concentrations of the grass samples after contact with AMD would suggest that a cation-anion exchange reaction did occur. An exchange of the H^+ ions in the AMD and the inorganic cations (M^+) associated with anions present in the grass, could be responsible for the increase in pH observed in synthetic AMD after grass addition. The addition of grass to the RD AMD did not achieve any significant or sustained decrease in H^+ concentration, which could be attributed to the more complex matrix and higher mineral acidity of the RD AMD which would require alternate experimental conditions to achieve a decrease in H^+ concentration. Variances in structural and physical composition between the different grass types were not consistent, and it was not possible to attribute the decrease in H^+ concentration in AMD to any compositional parameter. Using locally sourced lignocellulosic materials in passive remediation systems is vital to the successful design of a cost-effective and sustainable, remediation system. This work is important as it explores the suitability of grass types that are readily available from the immediate vicinity of the Mpumalanga coalfields, South Africa to increase the pH of contacted AMD at ambient temperature.

1. Acid mine drainage and remediation

Acid mine drainage (AMD) occurs when sulfide-bearing ores are exposed to oxygen and water resulting in the formation of sulfate, dissolved metals, and acidity. Secondary reactions then occur when the acidic water dissolves other metals that are present in the surrounding ore bodies resulting in an acidic run-off that is contaminated with a range of heavy metals (Gazea et al., 1996; McCarthy, 2011; Simate and

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Ndlovu, 2014). Gold and coal mining activities have led to the generation of large quantities of AMD in the Witwatersrand goldfields and Mpumalanga coalfields which has negatively impacted the surrounding environment (Gazea et al., 1996; Ighalo et al., 2022; Simate and Ndlovu, 2014; Tutu et al., 2008).

Treatment of AMD seeks to achieve water of neutral pH with the removal of sulfates (SO_4^{2-}), iron (Fe), and other metal(*oid*)s. Conventional AMD treatment typically comprises acid neutralisation using alkaline materials such as limestone, lime, sodium hydroxide or sodium carbonate. The increase in pH results in the subsequent precipitation of dissolved metal(*oid*)s present in the AMD. These processes generally have high operating costs as well as prohibitive waste disposal costs (Johnson and Hallberg, 2002, 2005,).

The use of bio-remediation technologies in AMD treatment processes may include several chemical and biological treatment mechanisms, such as microbially mediated redox reactions, chemical and enzymatic hydrolysis; phytoextraction, and biosorption (Gazea et al., 1996). A key biological AMD remediation process is the reduction of sulfates to sulfide using sulfate-reducing bacteria (SRB). These bacteria obtain energy by anaerobic respiration using sulfate as an electron acceptor and organic carbon, or hydrogen as an electron donor in a process called dissimilatory sulphate reduction (DSR) (Ayala-Parra et al., 2016).

$$SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2CO_2 + 2H_2O \quad (1)$$

Eq. (2) shows the reaction between the soluble sulfides formed in Eq. (1), with cationic metals ($Me^{2+)}$ present in AMD to precipitate as the metal sulfide (Ayala-Parra et al., 2016).

$$Me^{2+} + S^{2-} + 2H^+ \rightarrow MeS\downarrow \quad (2)$$

For a DSR system to be cost-effective, an inexpensive and sustainable carbon source is required.

2. Lignocellulose biomass

Lignocellulosic biomass is an abundant, renewable resource that can be derived from agricultural and forestry residues, pulp, garden, municipal wastes, as well as roadside vegetation (Demirbaş, 2001; Koppram et al., 2014; Petrova and Ivanova, 2010; Westensee et al., 2018). Ramla and Sheridan (2015) demonstrated that grass could be used as an electron donor source for DSR in the remediation of AMD and as a resource for biofuel production provided a suitable pre-treatment is developed to break down lignin and increase the cellulose surface area and render it amenable to enzymatic hydrolysis (Li et al., 2022; Ramla and Sheridan, 2015; Rezania et al., 2020).

Lignocellulosic materials are primarily composed of cellulose, hemicellulose, and lignin. The cellulose and hemicellulose materials could provide a source of monomeric sugars that could be used in the DSR process and/or to produce biofuels. These sugars can only be accessed once a pre-treatment process is performed to remove the lignin and disrupt the lignocellulosic structure (Burman et al., 2018; Lakaniemi et al., 2013). Cellulose and hemicellulose are the most abundant polymers in lignocellulosic biomass with expected concentrations of between 40 and 50 % and 20–30 % respectively. Lignin content is expected at between 10 and 25 % (Shahzadi et al., 2014).

Springer and Harris (1985), state that the addition of lignocellulosic material to a mineral acid solution results in a decrease in hydronium ion (H^+) concentration of the mineral acid. This decrease is attributed to an ion-exchange reaction where the H^+ ions in the acid solution are exchanged with the inorganic cations (M^+) that are associated with the anions present in the lignocellulosic material (Springer and Harris, 1985). The simplified reaction for the bound anions using wood to denote the lignocellulosic material is shown in Eq. (3).

$$wood - COO^-M^+ + H^+ \rightarrow wood - COO^-H^+ + M^+ \quad (3)$$

The main inorganic cations found in wood include: calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), and potassium (K^+) and the main inorganic anions include: sulfates (SO_4^{2-}), phosphates (PO_4^{3-}), and chlorides (Cl^-) (Springer and Harris, 1985).

This work investigated the physical and chemical composition of different grass types from the eastern Mpumalanga Coalfield region in South Africa to (a) establish the suitability of these grasses to support an increase in pH of AMD for potential use in AMD remediation processes; and (b) establish whether there was sufficient inorganic cation content in grass to support a similar ion-exchange reaction as observed in wood when added to AMD.

3. Sampling and classification of grass

A total of fourteen grass samples were collected from roadside areas close to coal operations, extending across the Witbank coal region from Springs to Belfast (Pone et al., 2007). The "Springs," G16 grass was purchased from an unspecified agricultural supplier in Springs and provided a sample from far western reaches of the Witbank coal region. The G15 Vetiver grass *(Chrysopgon zizanioides)*, was sourced from Mhlalanyoni, a farm just outside of White River in Mpumalanga and was included in this study because of previously reported success in phytoremediation of soils contaminated with heavy metals (Daraz et al., 2023; Kiiskila et al., 2019). The grass samples were marked as G1–G16, and grass types were classified into types by comparison with catalogued specimens at the Moss Herbarium, at the University of the Witwatersrand. Grass sampling locations were selected to include a representative spread across the Witbank coal region in Mpumalanga, (South Africa). Grass identification and sampling locations were superimposed on a map of the Mpumalanga region using the Microsoft Power BI package (Fig. 1).

4. Determination of the structural and chemical composition of grasses

The physical and chemical composition of the grasses were determined using various chemical and instrumental analytical techniques. Compositional and structural analysis of the grasses was conducted at the University of the Witwatersrand, Johannesburg's Schools of Chemical and Metallurgical Engineering, and Chemistry laboratory facilities.

5. Determination of extractives, structural carbohydrates, and lignin

Lignocellulosic biomass is primarily composed of cellulose, hemicellulose, and lignin. The relative proportion of each of these components was determined using the National Renewable Energy Laboratory (NREL) method (Sluiter et al., 2012). The removal of extractives or non-structural carbohydrates before compositional analysis was achieved using a single-stage Soxhlet extraction using acetone (Ayeni et al., 2015). The extractive-free grass was subjected to a two-stage hydrolysis process, requiring the addition of concentrated sulfuric acid with incubation at 30 °C for 1 h, followed by dilution and autoclaving at 121 °C for 1 h. The leached grass solids were separated from the solution using vacuum filtration. The acid-soluble lignin content was determined using a diluted portion of the filtrate solution and determining the absorbance spectrometrically at a wavelength of 320 nm, in a time-critical measurement that had to be made within 6 h of hydrolysis. Carbohydrates were determined by neutralising a portion of the filtrate solution to a pH range of between pH 5–6 with calcium carbonate and analysing the settled supernatant liquor after filtration through an 0.25 μm filter on an Agilent G1314B high-performance liquid chromatography (HPLC) instrument, with a 1200 series wavelength detector, using a Bio-Rad Animex HPX - 87H column coupled to a G1362A refractive index detector. Calibration standards for the carbohydrate determination were prepared using the same hydrolysis process as the samples. Acid-insoluble lignan was determined gravimetrically from the filter

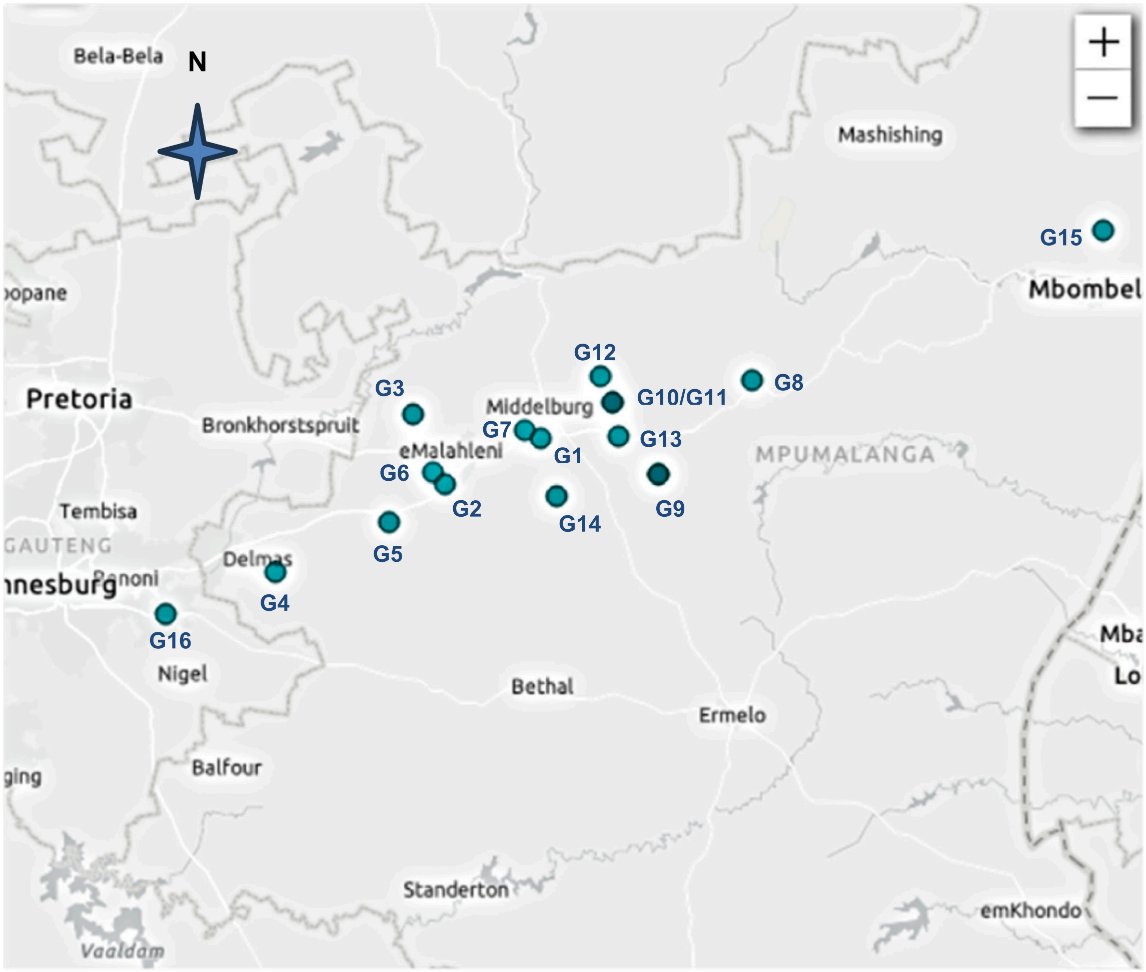

Fig. 1. The location of the grass samples collected in the Mpumalanga coalfields region of South Africa (Scale 1:2200000 cm).

residue after washing with deionised water and drying to constant mass at 105 °C.

6. Ultimate composition analysis

Carbon, hydrogen, nitrogen, and sulfur (CHNS) composition were determined using an Elementar, Vario EL cube CHNS analyser. Catalytic combustion was carried out at 1150 °C, and the evolved gas mixture components were separated using purge and trap chromatography, and measurements made by a thermal conductivity detector (TCD). The elemental concentrations were calculated by the instrument software and the oxygen content was determined by difference (Elementar Analysensysteme GmbH, 2023).

7. Proximate analysis

A thermo-gravimetric technique was used for the determination of moisture, volatile matter, fixed carbon, and ash content. The mass loss resulting from heating the sample to predefined temperatures under set conditions is proportional to the respective parameter, and the ash content was calculated by difference. For comparison, grass samples were analysed using two different programs, using an ALS SDT Q 600 TA instrument (TGA). The first method as described by Moyo (2013) required a heating rate of 30 °C/min in a nitrogen atmosphere of 40 mL/min to a temperature of 110 °C to determine the moisture content, and then to 700 °C to determine the volatiles content, before changing to an oxygen atmosphere, at the same flow rate and heating to 900 °C, to determine the carbon content (Moyo, 2013) In the second method described by Ayeni et al. (2015), the samples were held at 25 °C for 4 min in a nitrogen atmosphere, at a flow rate of 45 mL/min, the sample was then heated to 110 °C at a rate of 85 °C/min and held constant at this temperature for 5 min. The devolution step required heating to 950 °C at a rate of 80 °C/min, where the temperature was held constant for 5 min before changing to an oxygen atmosphere and maintaining these conditions for 7 min to achieve combustion of the remaining char. The ash content was calculated by subtraction of the percentage moisture, volatile matter, and fixed carbon content from 100 % (Ayeni et al., 2015; Moyo, 2013).

8. Calorific value analysis

The calorific value (CV) is a measure of the heat liberated when the grass is completely combusted in oxygen. An Energy Instrumentation, Supercal Modular Calorimeter was used for this determination, and the total heat energy released is reported in megajoules per kilogram (MJ/kg).

9. Determination of pH, oxidation-reduction potential (ORP), and dissolved oxygen (DO)

pH was measured using an Ohaus ST3–100 combined pH and mV meter and ST310 pH electrode with an integrated temperature probe. ORP measurements were made using an Ohaus ST3–100 combined pH and mV meter using a STORP1 ORP probe with a double junction Ag/AgCl reference. DO was measured using an Aqualitic AL 15 portable meter and oxygen sensor with automatic temperature compensation.

The suitability of using the grasses collected from the Mpumalanga coalfields region, (South Africa), for AMD remediation was tested by observing whether the addition of these grasses to both synthetic and RD AMD solutions, resulted in significant changes in pH. ORP and DO were also measured.

The RD AMD, (pH 2.2) was sourced from a coal mine in the eMalahleni region in Mpumalanga, (South Africa), and comprised ≈3200 mg/L Fe, ≈4800 mg/L total sulfur, 500 mg/L Ca, ≈400 mg/L Al and Mg, ≈80 mg/L Mn, ≈35 mg/L Na and Si, and between 1 and 10 mg/L of Co, Ni, and Zn (Smith et al., 2021). The synthetic AMD solution was prepared using Merck, analytical grade sulfuric acid and ferrous sulfate, to give a final concentration of 2500 mg/L of Fe and 5000 mg/L of sulfate. The solution pH was adjusted to ≈pH 4 using sodium hydroxide (NaOH) and made up to volume using deionised water (DI), Analytical grade reagents provide guaranteed purity, and as such, no other metal(*oid*)s were expected to be present in the synthetically prepared AMD. The metal(oid) and sulfate concentrations were not re-evaluated in this experimental work, as the purpose of this study was to assess the suitability of different grass types to increase the pH of AMD, for potential use in passive remediation systems.

10. Single grass addition experiment – procedure for sample and reactor preparation

Grasses were fed through a commercial garden shredder and then further reduced to size fractions of between 2 and 5 cm using a kitchen blender. The grasses were washed by rinsing 5 times with distilled water and dried over 3 days in a Labcon drying oven at 60 °C. Individual reactors were prepared using 750 mL Consol bottles. A 30 g/L grass:AMD ratio was achieved by weighing 15 g portions of grass into separate reactors and adding 500 mL of synthetic AMD. A further two reactors were prepared using RD AMD to test if these grass types reacted differently in different AMD media, and two control reactors were prepared using synthetic and RD AMD without grass addition. Only two grass types, G6 and G15 were contacted with RD AMD to preserve the limited quantities of RD AMD available for subsequent experimental work. The solutions were swirled to wet the grass completely and the bottles were sealed. pH, ORP and DO measurements were taken and recorded over eight days.

Six grass types were selected for further test work to establish if multiple additions of fresh grass to AMD, resulted in an incremental increase in pH. Three grasses from the previous, single grass addition experiment that demonstrated the highest increase in pH, and three different grass types were randomly selected to include for verification of previous data and comparison.

11. Multiple grass addition experiment - sample and reactor preparation

Samples were prepared in the same way as for the previous experiment. Duplicate portions of each grass sample were weighed into separate Consol bottles. RD AMD was added to the first grass portion and synthetic AMD was added to the second grass portion. A 30 g/L solid-to-liquid ratio was maintained throughout this experimental work, which was terminated after 10 days. Blank samples were included using RD water and synthetic AMD solutions. Immediately after reactor preparation and mixing, pH, ORP and DO were measured. These parameters were measured on day three and immediately after the grass was removed from the reactor solutions by filtration, and a second portion of fresh grass was added on day four. The same parameters were measured on the seventh day before removing the spent grass, and immediately after the addition of the third portion of fresh grass. The third portion of grass was removed on the ninth day and final measurements were taken on day 10 when the experiment was terminated. All spent grass was dried at 60 °C for 60 h, pulverized and passed through an 80 μm-mesh screen where the −80 μm fraction was retained for chemical analysis.

12. Chemical analysis of grass samples before and after contact with AMD

The grass samples retained from the second experiment were prepared for analysis by digesting in an Anton Paar Multiwave GO - Microwave digestion system, using a rapid digestion procedure described by Huang et al. (2004). The samples were made up to volume in a 1 % ultrapure nitric acid solution from which a ten-times dilution was made in the same 1 % ultrapure nitric acid before submitting to the School of Chemistry at the University of Johannesburg for chemical analysis. An inductively coupled plasma–optical emission spectrometry (ICP–OES) - Spectro Arcos (SOP) was used to analyse sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), phosphorus (P), and all sulfur-containing compounds as (S), while an inductively coupled plasma-mass spectrometry (ICP–MS), Perkin Elmer NexION 300D B was used to analyse aluminium (Al), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), and lead (Pb) (Havlin and Soltanpour, 1980; Huang et al., 2004). Quality control samples for these analyses included analysis of a reagent blank, a Tea Leaves certified reference material (CRM) and a duplicate analysis of the G15 (Vetiveria) grass sample. All sample and CRM data are presented in Appendix D, Table D.1, where averaged values of duplicate samples are reported with their standard deviations. Duplicate samples returned excellent precision, and duplicate G15, T2, and the Tea Leaves CRM samples reported relative standard deviations (RSD)s of less than 5 % for all elements. The G15, T0 duplicate samples, returned more varied RSDs, which could have resulted from digestion or instrumental inconsistencies; Na, Mg, K, Ca, S, B, and Zn returned RSDs of below 5 %, Mn and Cu returned RSDs of ≤10 %, and P, Al, Co and Pb returned RSDs over 14 %. The Tea Leave CRM returned concentrations within specified tolerances for most elements except for Na and Co. The elevated recovery for Na in the CRM material can be attributed to the reduced instrumental sensitivity for this element at low concentrations, and Co reported a marginally low biased concentration.

13. Grass classification

Grasses were identified by comparing the sampled grasses with specimen grasses catalogued at the Moss Herbarium at the University of the Witwatersrand, Johannesburg facilities. The grass site identification and grass classification are given in Appendix A, Table A.1.

Hyparrhenia hirta were identified individually at five of the sampling locations and mixed with other grasses at the G10 and G14 locations. *Eragrostis curvula* grass was identified at four locations, and the remaining grass varieties included, *Digitaria eriantha*, *Eragrostis plana*, *Hyparrhenia filipendula*, *Pogonarthria squarrosa*, and *Chrysopogon zizanioides* (Table 1).

14. Composition of grass

The average structural, proximate, CV, and ultimate, ranges were determined for all grass types at all locations are presented in Table 2 (Table 2).

Cellulose was the most abundant structural component in the grass samples analysed. An unusually low cellulose content of 22.5 % was reported for G14, while the remaining grasses reported cellulose content

Table 1
Grass name and classification.

Grass Type	Grass Site Identification
Hyparrhenia hirta	G2, G8, G9, **G10**, G12, G13, **G14**
Digitaria eriantha	G1
Eragrostis curvula	G4, G6, G7,G16
Chrysopogon zizanioides	G15
Pogonarthria squarrosa	G11
Eragrostis plana	G3
Hyparrhenia filipendula	G5

Table 2
The averaged structural, proximate, ultimate, and calorific value analysis concentration ranges of the collected grass samples.

Parameter (Structural)	Composition (%)	Parameter (TGA – Proximate) & (CV)	Composition TGA *(%)*, CV *(MJ/kg)*	Parameter (Ultimate)	Composition (%)
Cellulose	22.5–39.9	Moisture	2.3–4.1	T Carbon	32.6–47.4
Hemicellulose	20.9–25.2	Volatiles	58.9–81.3	T Sulfur	0.11–0.53
Lignin	17.5–22.5	Fixed C	14.8–19.1	Nitrogen	0.00–1.0
Extractives	2.1–6.7	Ash content	0.00–22.6	Hydrogen	5.0–6.6
		CV	13.7–19.3	Oxygen	45.7–61.5

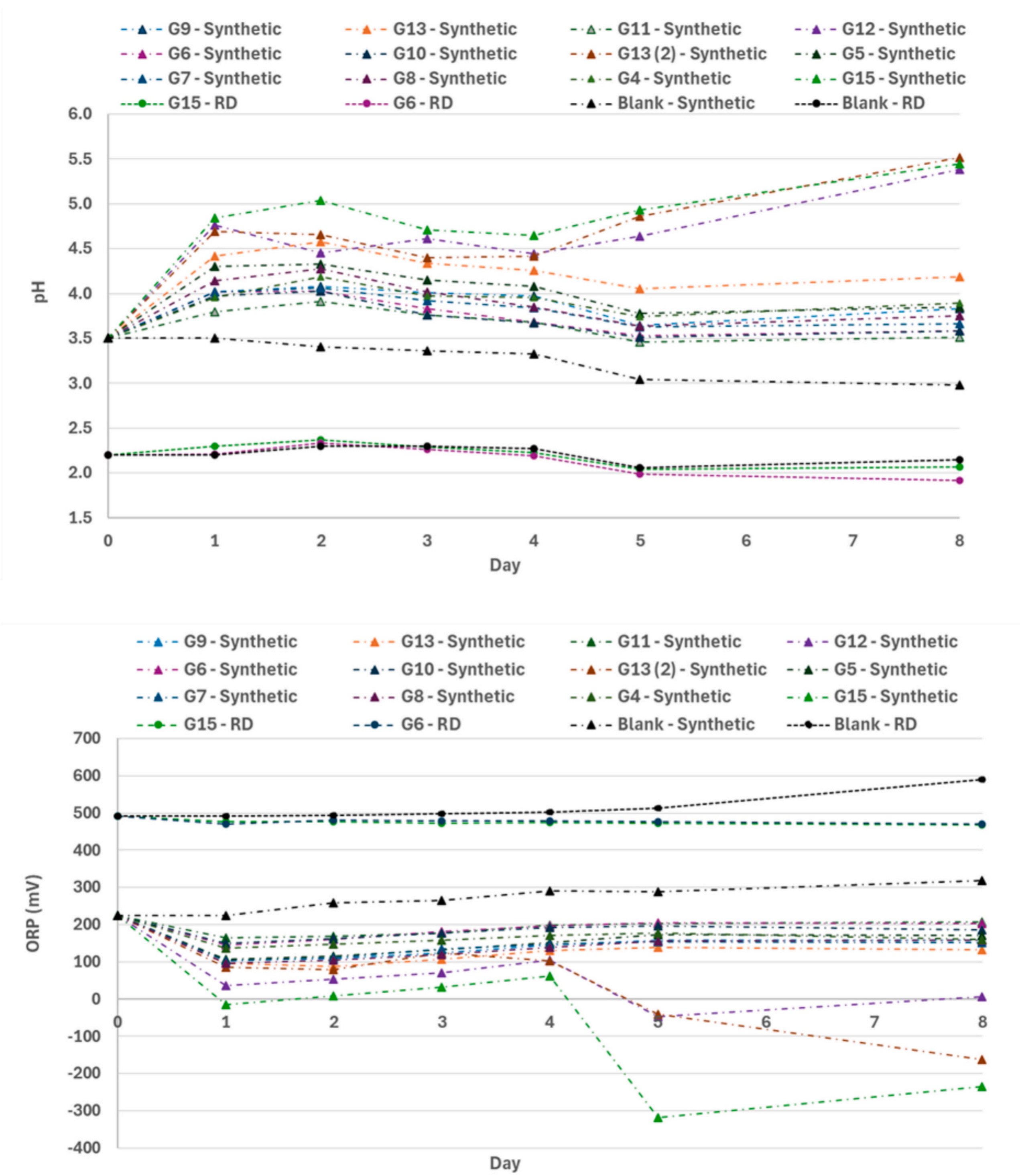

Fig. 2. pH and ORP data for synthetic and receiving dam (RD) AMD solutions after grass addition.

ranging between 28.6 and 39.9 %. The cellulose content reported lower average compositions than the 40–50 % as stated by Shahzadi et al. (2014), but within the 25–40 % range given for grasses by Malherbe and Cloete (2002). The 17–23 % lignin content and 21–25 % hemicellulose content were within the expected concentration range for agricultural biomass (Malherbe and Cloete, 2002; Shahzadi et al., 2014).

Proximate analysis data reported an average moisture content of between 2.3 and 4.1 % for all the grass samples. Except for the G14 grass which presented an unusually high ash content, the remaining grass samples presented a percentage ash content similar to the variations for weeping love grass, switchgrass, elephant grass and dwarf Napier grass, as reported by Burman et al. (2018).

The total carbon, total sulfur, hydrogen, and nitrogen content were analysed using an Elementar, Vario EL cube CHNS analyser, and the oxygen content was calculated by difference.

The averaged data and standard deviations for the individual grasses are given in Appendix A, Tables A.2, A.3 and A.4, respectively.

15. Single grass addition - determination of the suitability of grass for AMD remediation

This experiment intended to establish the suitability of different grass types collected from the Mpumalanga coalfields, South Africa, to aid AMD remediation by increasing the pH of contacted AMD. pH and ORP were selected as the test parameters to measure the changes in AMD over time. pH measurements would provide information on the H^+ concentration, while ORP readings would provide information on the AMDs' overall reducing or oxidising capacity. pH, ORP and DO data are used as control measurements in wastewater treatment systems, and ORP values provide a quick measurement of possible biological (oxidation-reduction) reactions occurring in treatment systems (Corominas et al., 2006; YSI Environmental, 2008). These data were recorded before and after the addition of each grass type to AMD. All grasses were tested using synthetic AMD, and G6 and G15 grass types were also tested using RD AMD. Dissolved oxygen (DO) readings were recorded from the second day of experimental work, and since the initial DO concentrations were not determined before grass addition, these data were not considered conclusive. The changes in pH observed for the synthetic and the RD samples are shown in Fig. 2. Synthetic sample data are represented as triangles, while the RD data are represented as circles.

The samples containing the synthetic *Hyparrhenia hirta* (G12 and (G13 (2)), and *Chrysopogon zizanioides* (G15) grass types presented the greatest initial increase in pH on the first day after grass addition increasing from a pH of 3.5 to an average of pH of 4.8. A significant increase in pH was observed after the fourth day for these samples reporting the highest final average pH of ≈5.5 on the eighth day after the termination of the experiment. The remaining samples containing synthetic AMD reported an increase of between 0.5 and 1.0 pH units on day one after the addition of grass, and marginal to no increase on the second day, followed by a decrease in pH until the termination of the experiment, reporting a final average pH of ≈3.7. The synthetic blank gradually decreased in pH for the duration of the experiment, while marginal changes were observed for the RD samples and any change observed in the sample H^+ concentration was also observed in the RD blank sample. Both synthetic and RD blank samples reported decreased pHs after the experiment. The drop in pH for the blank samples was expected because of the dissolution of CO_2 from the atmosphere into the AMD solution resulting in the formation of carbonic acid and, the probability of precipitation of ferric hydroxide at pH levels between pH 2.3 and 3.5, which removes ferric iron from solution resulting in the production of further hydrogen (H^+) ions with subsequent reduction of pH levels (Akcil and Koldas, 2006; Bowman et al., 2023).

ORP values for these samples varied throughout the experiment. The RD samples reported initial ORP values of 491 mV, and the synthetic samples reported initial ORP values of 224 mV. ORP changes of between 4 and 4.7 %, were observed for the RD samples, compared with the 7.5–205 % change in ORP for the synthetic samples. The changes in ORP for the synthetic samples and blanks generally inversely followed the trends observed in pH, with G12, G13 (2) and G15 samples showing a significant reduction in ORP between the fourth and fifth days, moving from positive to negative ORP values (Fig. 2). The decrease in ORP with the concomitant hydrogen ion (H^+) concentration decrease (pH increase), could be a result of a DSR reaction where electrons are donated from carbohydrates present in the grass resulting in the neutralisation of pH and consumption of H^+ (Bowman et al., 2023). This was however not confirmed experimentally, and the negative ORP readings were used as an indication of a possible biological process occurring (Environmental, Y.S.I, 2008). All pH, ORP, and DO data are presented in Appendix B, Tables B.1, B.2 and B.3 respectively.

16. Multiple grass addition - the effects of multiple additions of fresh grass to AMD on solution pH, ORP, and DO

Initial measurements were recorded on the first day before and after the addition of grass to the AMD samples. Measurements were taken on the fourth day after the removal of the spent grass and the addition of a second portion of fresh grass. Measurements were taken on the seventh day before and immediately after the addition of the third portion of fresh grass. The third portion of grass was removed on the ninth day after measuring parameters, and final measurements were taken on the tenth day before terminating the experiment. The pH and ORP data for the synthetic and RD samples are shown in Fig. 3. The vertical lines indicate the addition of fresh grass portions.

An increase in pH was observed for all synthetic samples immediately after each successive grass addition. The synthetic and RD blank samples showed an unexpected increase in pH after the second grass addition, indicating that the pH increase observed after the addition of this grass portion, could be attributed to instrumental error, despite the pH meter being calibrated before analysing the samples. This explanation is supported by the increased rather than the expected decrease in ORP associated with a pH increase (decrease in H^+ concentration). The changes in pH observed for the RD samples followed the trends observed in the RD blank sample, which would suggest that the grass did not achieve any notable change in pH in the RD samples. This testwork reported similar increases in pH for both G13 and G13 (2) synthetic samples, and the highest overall pH values were achieved for the G12 and G13 and G15 grasses. The replenishment of the fresh grass in the multiple grass addition experiment appeared to maintain the pH increase in the synthetic AMD samples for the duration of the experiment. The decrease in pH of the synthetic blank sample observed after the experiment would support the dissolution of CO_2 from the atmosphere with a concomitant increase in H^+ concentration (Bowman et al., 2023).

The RD samples reported an initial ORP of 489 mV which was similar to the initial ORP value for the single grass addition experiment, while the synthetic samples reported a higher initial ORP value of 321 mV compared with the 224 mV recorded for the single grass experiment. An anomalous reduction in ORP recorded for the RD blank on the seventh day, and an anomalous increase in ORP for the synthetic blank recorded on the eighth day, were probably owing to a sample swap, and these data were omitted from Fig. 3. The RD and synthetic blank samples demonstrated increased final ORP values, while all samples reported reduced final ORP values. Changes in ORP for the RD samples ranged between ≈1.8–6.5 %, compared with the ≈39.9–53.6 % range observed for the synthetic samples. The larger decrease in ORP observed in the synthetic AMD samples would support the more significant increase in pH observed for these samples (Bowman et al., 2023). Synthetic samples reported an initial DO concentration of 73 % compared with 29 % for the RD samples. Spurious data reported on day four for the synthetic G13, and day ten, for the RD G13 (2) samples could have been as a result of inadvertent analysis duplication of the respective preceding and subsequent samples. Except for the synthetic G15, G13 (2) and G12 samples, the DO concentration for all samples increased after the first grass

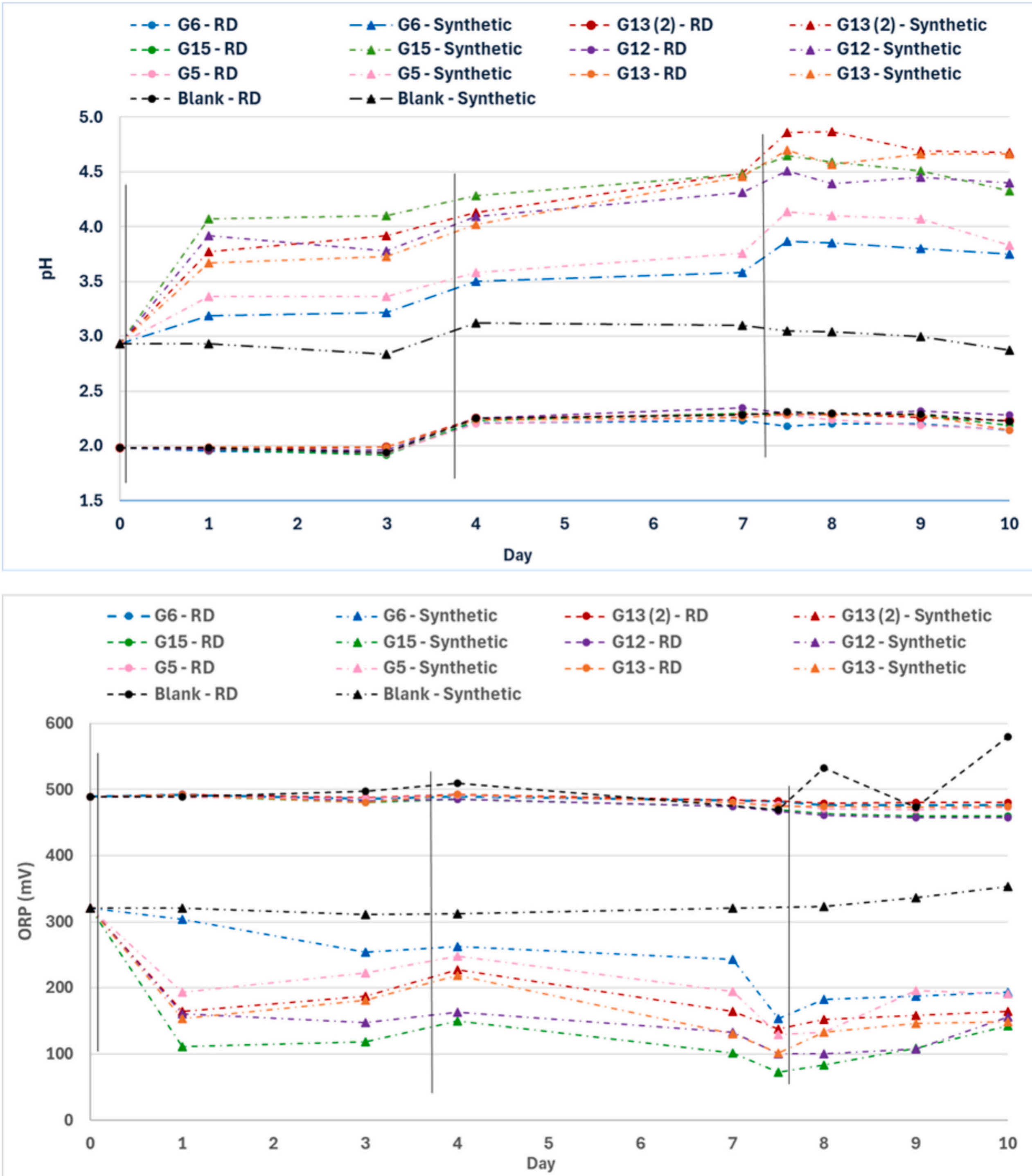

Fig. 3. pH and Eh data for synthetic and return dam (RD) AMD solutions after subsequent grass addition.

addition. A negligible change in DO concentration was observed after the second grass addition, and a pronounced increase in DO concentration was noted immediately after the third grass addition. The final DO concentration for synthetic samples reported between ≈4 and ≈13 %, and RD samples reported between ≈66 and ≈77 %. The depletion of DO concentration observed in the synthetic samples could be aligned with a DSR reaction where electrons are accepted by sulfate for microbial respiration, but this was not confirmed experimentally (Zhou et al., 2022). The behaviour of the blank samples cannot be explained, the synthetic blank sample DO concentration declined, from ≈70 % to ≈50 %, while the RD blank DO concentration declined from ≈30 % to ≈10 %, but then increased after the third grass addition, to report a final DO concentration of ≈50 %. The data for testwork conducted to determine whether the addition of fresh grass significantly affected the changes observed in pH in AMD samples are presented in Appendix C, Tables C.1, C.2 and C.3 for pH, ORP and DO, respectively.

17. Elemental concentration data for grass samples before and after subsequent addition to AMD

To explore whether the increase in pH could have resulted from an ion-exchange reaction between the hydronium ions in the AMD and the inorganic cations present in the grass (Springer and Harris, 1985), the relevant concentrations of cations and sulfur in the grass were determined before and after grass addition. Analysis was conducted using ICP:OES and ICP:MS after microwave digestion of selected grass samples. The elements present in the highest concentrations included K, S, Ca, Mg, P, Al and Na respectively, which were per the principal cation components in wood described by Springer and Harris (1985). In this instance, all sulfur compounds reported as S. Quality control measures included analysis of a sample blank, duplicate analysis of the original, the first addition of G15 grass samples, and analysis of a Tea Leaves certified reference material (CRM). Except for Na, which was present in low concentrations, the CRM concentrations returned values within the accepted tolerances. All sample and CRM data are presented in Appendix D Table D.1 where averaged values of duplicated samples were reported with their standard deviations. The poor CRM recovery for Na could be attributed to reduced instrumental sensitivity for Na at low concentrations. However, except for the low Na concentrations observed in the initial *Hyparrhenia filipendula* and *Eragrostis Curvula* grass samples, Na reported concentrations in orders of magnitude higher than the given CRM value. At these high concentrations, Na would not be subject to the instrumental error observed at method detection limits. The K, Mg, Ca, P, Na and S concentrations are shown in Fig. 4 where the first, second and third grass addition samples are denoted as Add 1, Add 2, and Add 3 respectively.

The *Hyparrhenia hirta* grass type reported the highest initial K concentrations, followed by *Chrysopogon zizanioides*, *Hyparrhenia filipendula*

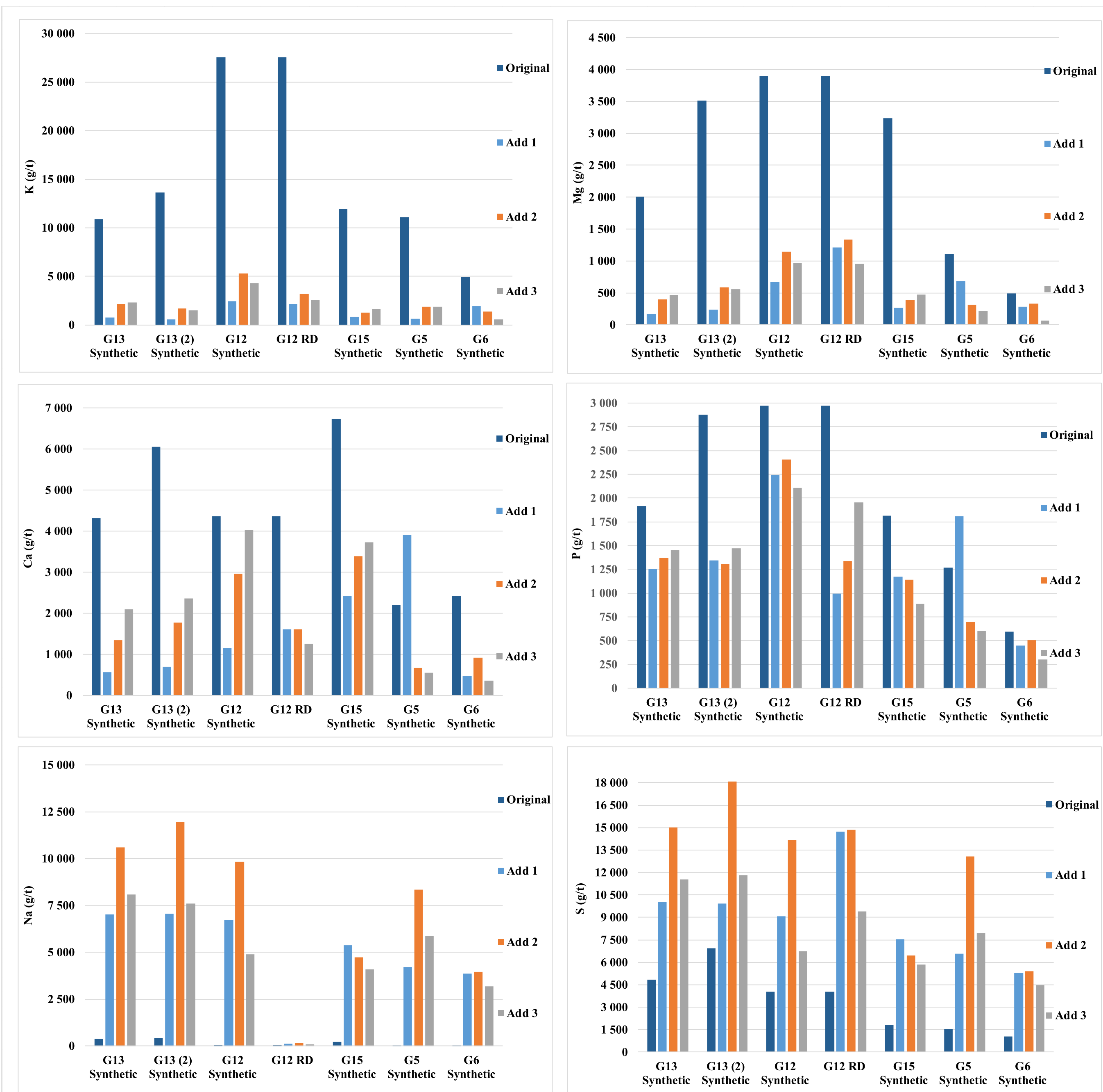

Fig. 4. K, Mg, Ca, P, Na and S concentrations in grass samples before and after subsequent addition to AMD. Samples 13, 13 (2), and 12 are classified as *Hyparrhenia hirta*, grass 15 is classified as *Chrysopogon zizanioides*, G5 as *Hyparrhenia filipendula*, and G6 as *Eragrostis Curvula*.

and *Eragrostis Curvula*. Decreased K concentrations were reported in all grass types after the addition of AMD.

The *Hyparrhenia hirta* and *Chrysopogon zizanioides* grass types reported the highest initial Mg concentrations, followed by *Hyparrhenia filipendula* and *Eragrostis Curvula* grass types which reported between 50 and 75 % lower Mg concentrations. Decreased Mg concentrations were reported in all grass types after the addition of AMD.

The *Chrysopogon zizanioides* and *Hyparrhenia hirta* grass types reported the highest initial Ca concentrations compared with the *Hyparrhenia filipendula* and *Eragrostis Curvula* grass types. All first-addition grasses reported decreased Ca concentrations except for an unexplained increase in Ca concentration that was observed for the first addition *Hyparrhenia filipendula* grass sample. Increased Ca concentrations were reported for the second- and most third-addition grasses. The third addition synthetic AMD *Hyparrhenia filipendula* and *Eragrostis Curvula* grass samples, and the *Hyparrhenia hirta* RD AMD grass samples reported decreased Ca concentrations.

The *Hyparrhenia hirta* and *Chrysopogon zizanioides* grass types reported the highest initial P concentrations, followed by *Hyparrhenia filipendula* and *Eragrostis Curvula* grass types. All first addition grasses reported decreased P concentrations except for an unexplained increase in P concentration that was observed for the first addition *Hyparrhenia filipendula* grass sample. An increase in P concentration was observed for the third grass addition for most *Hyparrhenia hirta* samples, while the remaining grass types showed decreased P concentrations in the third addition grasses.

All initial grass samples reported between one and two orders of magnitude lower Na concentrations compared with the Na concentrations reported for the first, second and third grass addition samples. Except for the synthetic *Eragrostis Curvula*, *Chrysopogon zizanioides*, and the RD *Hyparrhenia hirta* grass, the concentrations of Na in the second grass addition reported between 46 % – 97 % more than the first addition grasses. Decreased Na concentrations were observed for all third-addition samples, which, in most instances reported similar Na concentrations to the first-addition grass concentrations.

Despite initial S concentrations reporting at least one order of magnitude greater than initial Na concentrations, S concentrations followed the same trends observed for Na concentrations.

Conversion of logarithmic pH values to hydrogen ion (H^+) concentrations in mmol/L presented an increase in pH as a decrease in H^+ concentration. The H^+ concentration for the synthetic single and multiple grass experiments decreased while marginal to no decrease in H^+ concentration was observed in the multiple grass experiment. Any decrease in H^+ concentration observed for the RD samples was also observed in the RD blank, indicating that the addition of fresh grass did not achieve any significant reduction in H^+ concentration in these samples. The complex sample matrix and higher mineral acidity present in the RD AMD could explain why little to no reduction in H^+ concentration was observed in these samples (Smith et al., 2022).

The H^+ concentration data were used to calculate the percentage change in H^+ concentration after the initial grass addition, and the overall change in H^+ concentration compared with the initial H^+ concentration after each subsequent grass addition and at the termination of the single and multiple grass experiments. The percentage change in H^+ concentration for the synthetic AMD sample data is presented in Table 3.

The first grass addition for the multiple grass experiment yielded a smaller percentage decrease in H^+ concentration than was observed in the single grass addition experiment. This could be because of the elevated initial synthetic and RD AMD H^+ concentrations reported for the multiple grass experiment. The percentage decrease from initial H^+ concentration was enhanced with each successive grass addition in the multiple grass experiment and an improved overall percentage decrease in H^+ concentration was reported for the synthetic *Hyparrhenia hirta* (G13), *Eragrostis curvula* (G6), and *Hyparrhenia filipendula* (G5) samples. The *Chrysopogon zizanioides* (G15), *Hyparrhenia hirta* (G12 and G13 (2)) samples which performed best in the single grass experiment, reported similar percentage decreases in H^+ concentration after the multiple grass experiment.

Table 3
Percentage change in hydrogen ion concentration (H^+) from initial H^+ concentration after each successive grass addition to synthetic AMD, and after the experiment.

% Change in H^+ from before grass addition - Synthetic samples						
Grass ID	Single Grass - Day 1	Single Grass - Day 8	Multiple Grass - ADD 1	Multiple Grass - ADD 2	Multiple Grass - ADD 3	Multiple Grass removed - Day 8
G13	−88	−79.6	−81.8	−91.9	−98.3	−97.7
G13 (2)	−93.5	−99	−85.5	−93.7	−98.8	−98.9
G12	−94.5	−98.7	−89.8	−93.1	−97.4	−96.5
G15	−95.4	−98.9	−92.8	−95.5	−98.1	−97.8
G6	−69.8	−16.8	−45	−73.1	−88.5	−88.0
G5	−84.2	−55.3	−62.8	−77.6	−93.8	−93.2
Blank	0	231.1	0	−35.4	−24.1	−222.4

A decreased decline in percentage H^+ concentration was noted for the final readings taken one day after the removal of the last portion of grass in the multiple grass experiment and would suggest that grass addition did support the reduction in H^+ concentration. The *Hyparrhenia hirta* (G12 and G13), and *Chrysopogon zizanioides* (G15) grasses achieved the greatest percentage decrease in H^+ concentration and the lowest H^+ concentration (highest pH) in both single and multiple grass experiments. All H^+ data are shown in Appendix B, Tables 4–6 for the single grass experiments, and Appendix C, Tables 4–6 for the multiple grass experiments.

Except for the *Chrysopogon zizanioides* (G15), and *Hyparrhenia hirta* (G13 (2)) samples, the DO concentrations of the synthetic samples initially increased by between 4 % and 13 % in the multiple grass experiment. DO concentrations then decreased between 24 % and 79 % between the second and third days, reporting a final decrease in DO concentration of between 82 % – 94 % from the starting DO concentration. The RD samples reported a $\geq$ 150 % increase in DO concentration after the first grass addition. In most instances, a marginal increase in DO concentration was noted after each grass addition, which was generally followed by a reduction in DO concentration. The final increase in RD DO concentrations remained at $\geq$129 % of starting concentrations.

The decreasing DO concentrations observed over time in the synthetic grass samples could indicate the establishment of an anaerobic system over time, which is required to facilitate a dissimilatory reduction (DSR) process. This would be supported by the increased pH values observed and decreased ORP values for the synthetic samples, which could be attributed to the additional alkalinity produced from the reduction of sulfate in the system, which is further supported by the decrease in ORP observed (Ayala-Parra et al., 2016; Bowman et al., 2023). This can however not be confirmed, as the decrease in sulfate and metal(oid) concentration in the AMD was not determined as part of this experimental work.

The correlation between H^+ concentration and ORP was tested for each grass addition using the Excel data analysis tool at a 95 % confidence level. A strong positive correlation was observed after the first grass addition, reporting $r(11) = 0.85$, $p < .05$ and $r(5) = 0.92$, $p < .05$ for the synthetic AMD single and multiple grass experiments respectively. The correlation weakened to $r(11) = 0.65$, $p < .05$ on day eight at the end of the single grass experiment. In the multiple grass experiment, the correlation weakened to $r(5) = 0.85$, $p < .05$ after the addition of the second grass portion and then strengthened to $r(5) = 0.99$, $p < .05$ after the addition of the third grass portion on day seven. The correlation observed on day eight of the multiple grass experiment had weakened to $r(5) = 0.94$, $p < .05$ but remained stronger than the correlation reported on the eigth day for the single grass experiment. The H^+ concentration was positively correlated with the ORP, which confirms the negative correlation observed between pH and ORP (Bowman et al., 2023). A plot of the correlation which included all data for the duration of the single and multiple grass experiments (Fig. 5), using H^+ concentration as the independent (x) variable and the ORP value as the dependent (y) variable, gave a correlation coefficient (R^2) of 0.89 which was fit to a logarithmic curve for the single grass addition experiment, and a R^2 of 0.91 which was fit to a polynomial curve for the multiple grass experiment. Owing to the limited change in pH and ORP observed in the RD AMD, these samples generally produced poor H^+/ORP correlation.

Analysis of the major cations in the grass samples before and after addition to AMD showed the most pronounced decrease in K, Ca, and Mg concentrations observed in the first-addition grass, except for the *Hyparrhenia filipendula* (G5) first-addition grass which increased in Ca concentration. Again, except for the *Hyparrhenia filipendula* (G5), and *Eragrostis curvula* (G6) samples, these elements generally reported increased concentrations for second and third addition grasses. The K, Ca, and Mg concentrations for all grasses' third-addition grasses were lower than the original sample concentration. The decreased cation concentrations reported could be a result of ion exchange reactions occurring between the inorganic anions associated with the cations present in the grasses and the H^+ ions in the AMD, in a similar mechanism as suggested by Springer and Harris (1985) (Springer and Harris, 1985). A decrease in H^+ concentration would result in an increase in pH, which might have caused precipitation of Na_2SO_4 and thus explain the increased Na^+ and S^{2-} concentrations observed in the synthetic first, second and third grass addition samples. The additional sodium in the synthetic AMD samples was probably introduced from the sodium

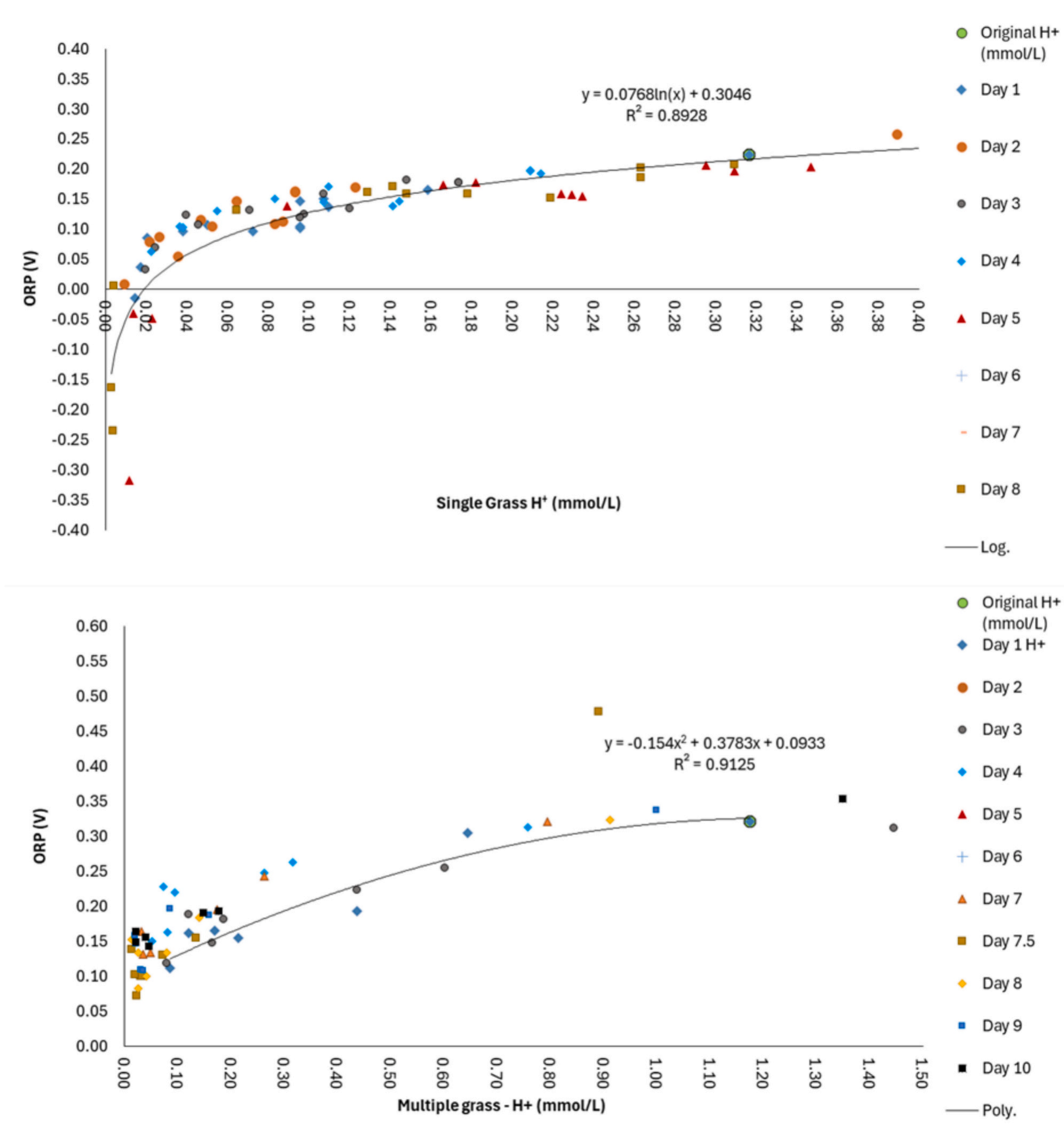

Fig. 5. Oxidation-reduction potential (ORP) plotted as a function of hydrogen ion concentration (H+) for single and multiple grass additions.

hydroxide (NaOH) used in the preparation of the synthetic AMD solution. No additional NaOH was introduced into the RD AMD sample, and previous analysis of this solution reported Na concentrations of less than 35 mg/L (Smith et al., 2021).

The best-performing grass types *Hyparrhenia hirta* (G12, G13) and *Chrysopogon zizanioides* (G15), reported higher moisture, extractives, total sulfur, oxygen, and lignin content, and the lower volatile, total carbon, Hydrogen, and CV content, than the *Eragrostis curvula* (G6), and *Hyparrhenia filipendula* (G5) samples. Percentage differences between the highest and the average concentration, and lowest and the average concentration for most components ranged between zero and 8.6 %. The most notable difference from the average was reported for *Hyparrhenia hirta* (G13) grass type which reported ≈154 % more than the average Total S concentration, and the *Hyparrhenia filipendula* (G5) grass type, which reported ≈88 % less than the average Total S concentration. The *Hyparrhenia filipendula* (G5) grass type reported 18.9 % less than the average moisture concentration. The variances from the average were not consistent for the best and worst performing grass types, making it difficult to assess whether compositional variances had any direct impact on the increase in pH of the synthetic AMD solutions.

The increase in the pH, or decrease in H^+ concentration, observed in all synthetic AMD solutions would indicate that the grass types tested could be considered suitable for use in AMD remediation. Multiple grass additions to synthetic AMD achieved a sustained and elevated percentage H^+ reduction for the duration of the experiment and facilitated a greater percentage H^+ reduction for the grass types *Eragrostis curvula* (G6), *Hyparrhenia filipendula* (G5) and *Hyparrhenia hirta (G 13)* that performed more poorly in the single grass addition experiment. The varying results achieved by the different grass types would indicate that some grass types would be more suited for AMD remediation. The *Hyparrhenia hirta* (G12, G13 (2)), and *Chrysopogon zizanioides* (G15) grass types achieved the greatest increase in pH in both the single and multiple grass experiments. A decrease in cation concentration of the grass samples from the multiple grass addition experiment could indicate the likelihood of a cation-anion exchange reaction between the H^+ ions in the AMD and the inorganic cations associated with the anions present in the grass. Single and multiple grass additions were, however, not successful in increasing the pH of the RD AMD samples under the given conditions. The varied structural and physical composition of the different grass types did not provide conclusive evidence on whether compositional parameters had any effect on the increase in pH.

Further test work should be conducted using different grass:AMD ratios and using aerobic and anaerobic conditions to establish if changing experimental parameters could achieve a greater decrease in H^+ concentration in RD AMD. Testwork should include the determination of all metal(*oid*)s, Fe, and sulfate concentrations to verify that a DSR process is occurring.

CRediT authorship contribution statement

Janet Smith: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Craig Sheridan:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Lizelle van Dyk:** Writing – review & editing, Supervision. **Kevin G. Harding:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

Appendix A. Appendix

Appendix A.1
Grass sample site identification and classification of grass samples collected in the Mpumalanga region.

Grass Site Identification	Grass Type
G1	*Digitaria eriantha steud. (Stapf.) Koler MS, J48205*
G2	*Hyparrhenia hirta (L.) Stapf, J064238*
G3	*Eragrostis plana. Nees, J40177*
G4	*Eragrostis curvula (Schrad.) Nees, J071222*
G5	*Hyparrhenia filipendula (Hochst.) Stapf* var. *pilosa (Hochst.) Stapf, J075391*
G6	*Eragrostis curvula (Schrad.) Nees, J071222*
G7	*Eragrostis curvula (Schrad.) Nees, J071222*
G8	*Hyparrhenia hirta (L.) Stapf, J064238*
G9	*Hyparrhenia hirta (L.) Stapf, J064238*
G10	*Possible Hyparrhenia hirta (L.) Stapf, J064238 & Cynodon prectostachyas curly (KSchum.)Pilg, J48725*
G11	*Pogonarthria squarrosa (Licha.) Pilg C.F. Clinning No. 42*
G12	*Hyparrhenia hirta (L.) Stapf, J064238*
G13	*Hyparrhenia hirta (L.) Stapf, J064238*
G14	*Possible Hyparrhenia hirta (L.) Stapf, J064238 & Hyparrhenia filipendula (Hochst.) Stapf var. pilosa (Hochst.) Stapf, J075391*
G15	*Vetiveria zinanioldes (Linn.) Nash, and reclassified as Chrysopogon zizanioides (L.) Roberty*
G16	*Eragrostis curvula (Schrad.) Nees, J071222*

Appendix A.2
Data for structural analysis of grass including extractives, lignin, cellulose, hemicellulose, and ash content.

Grass Site Identification	Grass Classification	Extractives (%)	Acid Soluble Lignin (%)	Acid insoluble Lignin (%)	Lignin (%)	Cellulose (%)	Hemicellulose (%)
G1	*Digitaria eriantha*	4.93 ± *1.33*	1.15 ± *0.16*	17.8 ± *1.53*	18.96	36.6 ± *3.10*	23.3 ± *1.87*
G2	*Hyparrhenia hirta*	2.60 ± *1.02*	1.01 ± *0.03*	17.1 ± *0.39*	18.09	32.8 ± *1.65*	22.4 ± *2.71*
G3	*Eragrostis plana*	3.79 ± *0.95*	1.71 ± *0.06*	16.3 ± *0.58*	17.96	25.7 ± *3.46*	22.2 ± *2.48*
G4	*Eragrostis curvula*	3.47 ± *1.43*	1.31 ± *0.07*	19.2 ± *0.23*	20.48	33.3 ± *2.62*	28.3 ± *2.68*
G5	*Hyparrhenia filipendula*	2.65 ± *0.38*	1.15 ± *0.07*	17.6 ± *0.74*	18.74	34.6 ± *5.56*	20.9 ± *0.92*
G6	*Eragrostis curvula*	4.57 ± *1.37*	1.10 ± *0.12*	16.7 ± *0.85*	17.75	34.9 ± *3.98*	21.9 ± *0.81*
G7	*Eragrostis curvula*	2.06 ± *0.79*	1.49 ± *0.19*	19.7 ± *4.61*	21.20	33.4 ± *4.87*	24.7 ± *3.58*
G8	*Hyparrhenia hirta*	3.91 ± *2.83*	1.34 ± *0.18*	23.4 ± *5.05*	24.71	28.4 ± *4.32*	23.0 ± *1.71*
G9	*Hyparrhenia hirta*	4.17 ± *2.51*	1.27 ± *0.05*	18.0 ± *0.55*	19.25	31.4 ± *3.80*	21.4 ± *0.92*
G10	*Hyparrhenia hirta / Cynodon prectostachyas curly*	5.42 ± *2.31*	1.55 ± *0.17*	18.9 ± *2.75*	20.49	31.6 ± *6.16*	20.9 ± *1.05*
G11	*Pogonarthria squarrosa*	5.16 ± *1.12*	1.69 ± *0.10*	20.9 ± *3.94*	22.55	28.2 ± *4.78*	22.3 ± *0.80*
G12	*Hyparrhenia hirta*	5.46 ± *2.80*	1.41 ± *0.04*	17.8 ± *0.63*	19.24	29.4 ± *4.46*	21.4 ± *0.81*
G13	*Hyparrhenia hirta*	6.05 ± *3.22*	1.66 ± *0.18*	17.7 ± *0.59*	19.33	28.6 ± *1.18*	23.3 ± *2.59*
G14	*Hyparrhenia hirta / Hyparrhenia filipendula*	5.16 ± *0.93*	1.74 ± *0.12*	16.6 ± *0.61*	18.30	22.5 ± *0.71*	25.2 ± *4.04*
G15	*Chrysopogon zizanioides*	4.80 ± *2.01*	1.26 ± *0.14*	17.5 ± *0.49*	18.80	32.6 ± *2.81*	25.2 ± *2.38*
G16	*Eragrostis curvula*	5.97 ± *2.01*	2.01 ± *0.19*	15.5 ± *0.38*	17.53	30.3 ± *4.18*	23.0 ± *2.54*

Appendix A.3
Data for Proximate analysis of grass including extractives, lignin, cellulose, hemicellulose, ash content and calorific value.

Grass Site Identification	Grass Classification	Moisture (%)	Volatile Matter (%)	Fixed Carbon (%)	Ash Content TGA (%)	Average CV (MJ/kg)
G1	*Digitaria eriantha*	2.92 ± *1.59*	79.6 ± *2.81*	16.2 ± *0.94*	1.25 ± *0.28*	17.6 ± *0.22*
G2	*Hyparrhenia hirta*	3.83 ± *0.32*	77.5 ± *0.55*	16.2 ± *1.78*	2.40 ± *1.55*	17.7 ± *0.76*
G3	*Eragrostis plana*	3.81 ± *0.28*	76.2 ± *0.66*	15.1 ± *1.27*	4.83 ± *0.34*	16.8 ± *0.08*
G4	*Eragrostis curvula*	4.07 ± *0.41*	71.3 ± *1.53*	17.0 ± *1.68*	7.61 ± *0.56*	16.7 ± *0.77*
G5	*Hyparrhenia filipendula*	2.65 ± *0.28*	76.8 ± *1.62*	18.5 ± *1.35*	2.07 ± *0.01*	17.5 ± *0.23*
G6	*Eragrostis curvula*	2.73 ± *0.24*	76.3 ± *1.06*	16.6 ± *1.32*	4.38 ± *0.50*	17.5 ± *1.36*
G7	*Eragrostis curvula*	2.27 ± *1.42*	81.3 ± *0.73*	17.3 ± *1.59*	0.35 ± *0.50*	18.2 ± *1.36*
G8	*Hyparrhenia hirta*	3.92 ± *0.06*	76.4 ± *1.73*	16.5 ± *1.73*	3.11 ± *0.05*	17.0 ± *0.06*
G9	*Hyparrhenia hirta*	3.33 ± *0.24*	74.3 ± *2.26*	17.9 ± *1.47*	4.57 ± *0.54*	17.4 ± *0.18*
G10	*Hyparrhenia hirta / Cynodon prectostachyas curly*	3.02 ± *0.37*	75.6 ± *2.39*	17.1 ± *2.07*	4.28 ± *0.05*	17.2 ± *0.03*
G11	*Pogonarthria squarrosa*	4.01 ± *0.67*	77.7 ± *0.66*	15.3 ± *1.78*	3.02 ± *0.45*	17.3 ± *0.09*
G12	*Hyparrhenia hirta*	2.88 ± *0.39*	74.1 ± *2.05*	16.3 ± *1.87*	6.79 ± *0.21*	16.8 ± *0.22*
G13	*Hyparrhenia hirta*	2.93 ± *0.05*	72.4 ± *2.59*	17.0 ± *2.47*	7.71 ± *0.16*	16.7 ± *0.23*
G14	*Hyparrhenia hirta / Hyparrhenia filipendula*	3.67 ± *0.63*	58.9 ± *2.25*	14.8 ± *1.53*	22.60 ± *0.10*	13.7 ± *0.05*
G15	*Chrysopogon zizanioides*	3.54 ± *0.15*	75.6 ± *0.03*	19.1 ± *1.61*	1.75 ± *1.50*	17.0 ± *0.10*
G16	*Eragrostis curvula*	2.48 ± *1.29*	80.9 ± *2.54*	17.2 ± *0.75*	0.00 ± *0.00*	19.3 ± *0.04*

Appendix A.4
Data for Ultimate analysis of grass including total carbon, hydrogen, nitrogen, total sulfur, and oxygen content.

Grass Site Identification	Grass Classification	Total Carbon (%)	Hydrogen (%)	Nitrogen (%)	Total Sulfur (%)	Oxygen - calculated (%)
G1	*Digitaria eriantha*	44.6	6.35	0.11	0.23	48.8
G2	*Hyparrhenia hirta*	42.8	6.1	0.0	0.3	50.9
G3	*Eragrostis plana*	41.8	6.1	0.5	0.4	51.2
G4	*Eragrostis curvula*	39.8	5.7	0.5	0.3	53.8
G5	*Hyparrhenia filipendula*	41.8 ± *0.85*	5.79 ± *0.11*	0.38 ± *0.0*	0.13 ± *0.01*	52.3
G6	*Eragrostis curvula*	41.7	5.8	0.0	0.1	52.3
G7	*Eragrostis curvula*	47.4	6.6	0.2	0.2	45.7
G8	*Hyparrhenia hirta*	39.8	5.7	0.3	0.2	54.0
G9	*Hyparrhenia hirta*	42.4	5.9	0.4	0.1	51.2
G10	*Hyparrhenia hirta / Cynodon prectostachyas curly*	41.3	5.7	0.0	0.1	52.8
G11	*Pogonarthria squarrosa*	39.9	5.7	0.3	0.2	53.9
G12	*Hyparrhenia hirta*	39.5	5.5	0.3	0.2	54.5
G13	*Hyparrhenia hirta*	38.2	5.5	1.0	0.5	54.8
G14	*Hyparrhenia hirta / Hyparrhenia filipendula*	32.6 ± *1.07*	5.01 ± *0.07*	0.69 ± *0.07*	0.23 ± *0.01*	61.5
G15	*Chrysopogon zizanioides*	41.1	5.7	0.5	0.1	52.5
G16	*Eragrostis curvula*	44.7	6.2	0.7	0.1	48.2

Appendix B. Appendix

Appendix B.1
pH data for single grass addition test work to establish the suitability of grass for AMD remediation.

Grass Site Identification	Grass Classification	Original pH	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
G9 – Synthetic	*Hyparrhenia hirta*	3.50	4.02	4.08	4.01	3.97	3.65	–	–	3.83
G13 Synthetic	*Hyparrhenia hirta*	3.50	4.42	4.58	4.34	4.26	4.05	–	–	4.19
G11 – Synthetic	*Pogonarthria squarrosa*	3.50	3.80	3.91	3.76	3.68	3.46	–	–	3.51
G12 – Synthetic	*Hyparrhenia hirta*	3.50	4.76	4.45	4.61	4.44	4.64	–	–	5.38
G6 – Synthetic	*Eragrostis curvula*	3.50	4.02	4.03	3.83	3.68	3.53	–	–	3.58
G10 – Synthetic	*Possible Hyparrhenia hirta & Cynodon prectostachyas curly*	3.50	3.97	4.03	3.76	3.67	3.51	–	–	3.58
G13 (2) – Synthetic	*Hyparrhenia hirta*	3.50	4.69	4.66	4.40	4.42	4.86	–	–	5.52
G5 – Synthetic	*Hyparrhenia filipendula*	3.50	4.30	4.33	4.15	4.08	3.78	–	–	3.85
G7 – Synthetic	*Eragrostis curvula*	3.50	4.02	4.06	3.92	3.84	3.63	–	–	3.66
G8 – Synthetic	*Hyparrhenia hirta*	3.50	4.14	4.28	4.02	3.85	3.64	–	–	3.75
G4 – Synthetic	*Eragrostis curvula*	3.50	3.96	4.19	3.97	3.96	3.74	–	–	3.89
G15 – Synthetic	*Chrysopogon zizanioides*	3.50	4.84	5.04	4.71	4.65	4.93	–	–	5.45
G15 – RD	*Chrysopogon zizanioides*	2.20	2.30	2.37	2.29	2.23	2.04	–	–	2.07
G6 – RD	*Hyparrhenia hirta*	2.20	2.21	2.33	2.26	2.19	1.99	–	–	1.92
Blank – Synthetic	*Blank – No grass*	3.50	3.50	3.41	3.36	3.33	3.04	–	–	2.98
Blank – RD	*Blank – No grass*	2.20	2.20	2.3	2.3	2.27	2.06	–	–	2.15

Appendix B.2
Oxidation-reduction potential data for single grass addition testwork to establish the suitability of grass for AMD remediation.

Grass Site Identification	Grass Classification	Original ORP (mV)	Day 1 (mV)	Day 2 (mV)	Day 3 (mV)	Day 4 (mV)	Day 5 (mV)	Day 6 (mV)	Day 7 (mV)	Day 8 (mV)
G9 – Synthetic	*Hyparrhenia hirta*	224	104	109	125	145	158	–	–	158
G13 – Synthetic	*Hyparrhenia hirta*	224	96	87	107	130	139	–	–	132
G11 – Synthetic	*Pogonarthria squarrosa*	224	165	169	178	198	203	–	–	207
G12 – Synthetic	*Hyparrhenia hirta*	224	37	54	70	104	−48	–	–	6
G6 – Synthetic	*Eragrostis curvula*	224	146	161	181	196	206	–	–	202
G10 – Synthetic	*Possible Hyparrhenia hirta & Cynodon prectostachyas curly*	224	150	163	178	193	197	–	–	186
G13 (2) – Synthetic	*Hyparrhenia hirta*	224	85	79	123	103	−40	–	–	−163
G5 – Synthetic	*Hyparrhenia filipendula*	224	107	115	132	151	174	–	–	171
G7 – Synthetic	*Eragrostis curvula*	224	102	112	134	147	154	–	–	152
G8 – Synthetic	*Hyparrhenia hirta*	224	96	105	120	138	157	–	–	159
G4 – Synthetic	*Eragrostis curvula*	224	137	147	158	171	177	–	–	161
G15 – Synthetic	*Chrysopogon zizanioides*	224	−15	9	33	63	−318	–	–	−235
G15 – RD	*Chrysopogon zizanioides*	491	476	476	473	474	472	–	–	468
G6 – RD	*Hyparrhenia hirta*	491	470	480	478	478	477	–	–	471
Blank – Synthetic	*Blank – No grass*	224	224	258	265	290	288	–	–	318
Blank – Plant	*Blank – No grass*	491	491	493	497	503	512	–	–	590

Appendix B.3
Dissolved oxygen data for single grass addition test work to establish the suitability of grass for AMD remediation.

Grass Site Identification	Grass Classification	Original DO	Day 1	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)	Day 6 (%)	Day 7 (%)	Day 8 (%)
G9 – Synthetic	*Hyparrhenia hirta*	–	–	21.9	11.0	18.2	20.0	–	–	22.0
G13 – Synthetic	*Hyparrhenia hirta*	–	–	7.2	13.2	16.8	8.5	–	–	10.0
G11 – Synthetic	*Pogonarthria squarrosa*	–	–	21.1	20.3	23.4	15.2	–	–	3.3
G12 – Synthetic	*Hyparrhenia hirta*	–	–	34.1	12.8	14.8	8.9	–	–	52.1
G6 – Synthetic	*Eragrostis curvula*	–	–	22.5	16.9	18.7	28.1	–	–	18.7
G10 – Synthetic	*Possible Hyparrhenia hirta & Cynodon prectostachyas curly*	–	–	11.7	9.2	21.2	10.2	–	–	7.6
G13 (2) – Synthetic	*Hyparrhenia hirta*	–	–	64.6	55.1	56.6	12.8	–	–	5.3
G5 – Synthetic	*Hyparrhenia filipendula*	–	–	9.4	27.0	18.7	28.0	–	–	22.4
G7 – Synthetic	*Eragrostis curvula*	–	–	53.2	39.8	14.9	12.7	–	–	13.2
G8 – Synthetic	*Hyparrhenia hirta*	–	–	28.3	25.3	19.3	23.3	–	–	6.7
G4 – Synthetic	*Eragrostis curvula*	–	–	9.9	34.3	23.0	38.2	–	–	17.8
G15 – Synthetic	*Chrysopogon zizanioides*	–	–	12.1	6.8	9.3	0.0	–	–	0.4
G15 – RD	*Chrysopogon zizanioides*	–	–	24.3	25.4	27.0	35.5	–	–	53.0
G6 – RD	*Hyparrhenia hirta*	–	–	56.7	66.7	64.2	57.3	–	–	61.1
Blank – Synthetic	*Blank – No grass*	–	–	64.5	64.8	65.2	69.9	–	–	68.5
Blank – Plant	*Blank – No grass*	–	–	61.5	16.6	15.6	16.4	–	–	46.4

Appendix B.4
Hydrogen ion (H^{+}) data for single grass addition testwork to establish the suitability of grass for AMD remediation.

Grass Site Identification	Grass Classification	Original H+ (mmol/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
G9 – Synthetic	*Hyparrhenia hirta*	0.32	0.10	0.08	0.10	0.11	0.22	–	–	0.15
G13 – Synthetic	*Hyparrhenia hirta*	0.32	0.04	0.03	0.05	0.05	0.09	–	–	0.06
G11 – Synthetic	*Pogonarthria squarrosa*	0.32	0.16	0.12	0.17	0.21	0.35	–	–	0.31
G12 – Synthetic	*Hyparrhenia hirta*	0.32	0.02	0.04	0.02	0.04	0.02	–	–	0.00
G6 – Synthetic	*Eragrostis curvula*	0.32	0.10	0.09	0.15	0.21	0.30	–	–	0.26
G10 – Synthetic	*Possible Hyparrhenia hirta & Cynodon prectostachyas curly*	0.32	0.11	0.09	0.17	0.21	0.31	–	–	0.26
G13 (2) – Synthetic	*Hyparrhenia hirta*	0.32	0.02	0.02	0.04	0.04	0.01	–	–	0.00
G5 – Synthetic	*Hyparrhenia filipendula*	0.32	0.05	0.05	0.07	0.08	0.17	–	–	0.14
G7 – Synthetic	*Eragrostis curvula*	0.32	0.10	0.09	0.12	0.14	0.23	–	–	0.22
G8 – Synthetic	*Hyparrhenia hirta*	0.32	0.07	0.05	0.10	0.14	0.23	–	–	0.18
G4 – Synthetic	*Eragrostis curvula*	0.32	0.11	0.06	0.11	0.11	0.18	–	–	0.13
G15 – Synthetic	*Chrysopogon zizanioides*	0.32	0.01	0.01	0.02	0.02	0.01	–	–	0.00
G15 – RD	*Chrysopogon zizanioides*	6.31	5.01	4.27	5.13	5.89	9.12	–	–	8.51
G6 – RD	*Hyparrhenia hirta*	6.31	6.17	4.68	5.50	6.46	10.23	–	–	12.02
Blank – Synthetic	*Blank – No grass*	0.32	0.32	0.39	0.44	0.47	0.91	–	–	1.05
Blank – RD	*Blank – No grass*	6.31	6.31	5.01	5.01	5.37	8.71	–	–	7.08

Appendix B.5
Percentage change in hydrogen ion concentration (H+) between days for single grass addition testwork.

Grass Site Identification	Grass Classification	% initial change in H^{+} (Day 0–1)	% change in H^{+} (Day 1–2)	% change in H^{+} (Day 2–3)	% change in H^{+} (Day 3–4)	% change in H^{+} (Day 4–5)	% change in H^{+} (Day 5–8)
G9 – Synthetic	*Hyparrhenia hirta*	−69.8	−12.9	17.5	9.6	108.9	−33.9
G13 – Synthetic	*Hyparrhenia hirta*	−88.0	−30.8	73.8	20.2	62.2	−27.6
G11 – Synthetic	*Pogonarthria squarrosa*	−49.9	−22.4	41.3	20.2	66.0	−10.9
G12 – Synthetic	*Hyparrhenia hirta*	−94.5	104	−30.8	47.9	−36.9	−81.8
G6 – Synthetic	*Eragrostis curvula*	−69.8	−2.3	58.5	41.3	41.3	−10.9
G10 – Synthetic	*Possible Hyparrhenia hirta & Cynodon prectostachyas curly*	−66.1	−12.9	86.2	23.0	44.5	−14.9
G13 (2) – Synthetic	*Hyparrhenia hirta*	−93.5	7.2	82.0	−4.5	−63.7	−78.1
G5 – Synthetic	*Hyparrhenia filipendula*	−84.2	−6.7	51.4	17.5	99.5	−14.9
G7 – Synthetic	*Eragrostis curvula*	−69.8	−8.8	38.0	20.2	62.2	−6.7
G8 – Synthetic	*Hyparrhenia hirta*	−77.1	−27.6	82.0	47.9	62.2	−22.4
G4 – Synthetic	*Eragrostis curvula*	−65.3	−41.1	66.0	2.3	66.0	−29.2
G15 – Synthetic	*Chrysopogon zizanioides*	−95.4	−36.9	113.8	14.8	−47.5	−69.8
G15 – RD	*Chrysopogon zizanioides*	−20.6	−14.9	20.2	14.8	54.9	−6.7
G6 – RD	*Hyparrhenia hirta*	−2.3	−24.1	17.5	17.5	58.5	17.5
Blank – Synthetic	*Blank – No grass*	0.0	23.0	12.2	7.2	95.0	14.8
Blank – RD	*Blank – No grass*	0.0	−20.6	0.0	7.2	62.2	−18.7

Appendix B.6
Percentage change in hydrogen ion concentration (H+) compared with initial H+ concentration for single grass addition testwork.

Grass Site Identification	Grass Classification	% initial change in H^{+} (Day 0–1)	% change in H^{+} (Day 0–2)	% change in H^{+} (Day 0–3)	% change in H^{+} (Day 0–4)	% change in H^{+} (Day 0–5)	% change in H^{+} (Day 0–8)
G9 – Synthetic	*Hyparrhenia hirta*	−69.8	−73.7	−69.1	−66.1	−29.2	−53.2
G13 – Synthetic	*Hyparrhenia hirta*	−88.0	−91.7	−85.5	−82.6	−71.8	−79.6
G11 – Synthetic	*Pogonarthria squarrosa*	−49.9	−61.1	−45.0	−33.9	9.6	−2.3
G12 – Synthetic	*Hyparrhenia hirta*	−94.5	−88.8	−92.2	−88.5	−92.8	−98.7
G6 – Synthetic	*Eragrostis curvula*	−69.8	−70.5	−53.2	−33.9	−6.7	−16.8
G10 – Synthetic	*Possible Hyparrhenia hirta & Cynodon prectostachyas curly*	−66.1	−70.5	−45.0	−32.4	−2.3	−16.8
G13 (2) – Synthetic	*Hyparrhenia hirta*	−93.5	−93.1	−87.4	−88.0	−95.6	−99.0
G5 – Synthetic	*Hyparrhenia filipendula*	−84.2	−85.2	−77.6	−73.7	−47.5	−55.3
G7 – Synthetic	*Eragrostis curvula*	−69.8	−72.5	−62.0	−54.3	−25.9	−30.8
G8 – Synthetic	*Hyparrhenia hirta*	−77.1	−83.4	−69.8	−55.3	−27.6	−43.8
G4 – Synthetic	*Eragrostis curvula*	−65.3	−79.6	−66.1	−65.3	−42.5	−59.3
G15 – Synthetic	*Chrysopogon zizanioides*	−95.4	−97.1	−93.8	−92.9	−96.3	−98.9
G15 – RD	*Chrysopogon zizanioides*	−20.6	−32.4	−18.7	−6.7	44.5	34.9
G6 – RD	*Hyparrhenia hirta*	−2.3	−25.9	−12.9	2.3	62.2	90.5
Blank – Synthetic	*Blank – No grass*	0.0	23.0	38.0	47.9	188.4	231.1
Blank – RD	*Blank – No grass*	0.0	−20.6	−20.6	−14.9	38.0	12.2

Appendix C. Appendix

Appendix C.1
pH data for multiple grass addition test work to establish whether incremental addition of fresh grass significantly affected the change in pH of the AMD.

Grass Site Identification	Grass Classification	Day 0 Original pH	Day 1 After 1st grass addition	Day 3	Day 4 After 2nd grass addition	Day 7	Day 7.5 After 3rd grass addition	Day 8	Day 9	Day 10 1 day after 3rd grass removal
G6 – RD	*Eragrostis Curvula*	1.98	1.95	1.93	2.21	2.23	2.18	2.20	2.20	2.14
G6 – Synthetic	*Eragrostis Curvula*	2.93	3.19	3.22	3.50	3.58	3.87	3.85	3.80	3.75
G13 (2) – RD	*Hyparrhenia hirta*	1.98	1.97	1.99	2.25	2.29	2.29	2.29	2.26	2.23
G13 (2) – Synthetic	*Hyparrhenia hirta*	2.93	3.77	3.92	4.13	4.49	4.86	4.87	4.69	4.68
G15 – RD	*Chrysopogon zizanioides*	1.98	1.97	1.92	2.23	2.30	2.28	2.30	2.28	2.19
G15 – Synthetic	*Chrysopogon zizanioides*	2.93	4.07	4.10	4.28	4.48	4.65	4.59	4.51	4.33
G12 – RD	*Hyparrhenia hirta*	1.98	1.97	1.96	2.25	2.35	2.30	2.28	2.32	2.28
G12 – Synthetic	*Hyparrhenia hirta*	2.93	3.92	3.78	4.09	4.31	4.51	4.39	4.45	4.40
G5 – RD	*Hyparrhenia filipendula*	1.98	1.99	1.93	2.20	2.26	2.28	2.24	2.19	2.15
G5 – Synthetic	*Hyparrhenia filipendula*	2.93	3.36	3.36	3.58	3.76	4.14	4.10	4.07	3.83
G13 – RD	*Hyparrhenia hirta*	1.98	1.99	1.99	2.24	2.26	2.29	2.28	2.28	2.14
G13 – Synthetic	*Hyparrhenia hirta*	2.93	3.67	3.73	4.02	4.46	4.70	4.57	4.66	4.66
Blank – RD	*Blank – No grass*	1.98	1.98	1.94	2.25	2.28	2.31	2.30	2.29	2.22
Blank – Synthetic	*Blank – No grass*	2.93	2.93	2.84	3.12	3.10	3.05	3.04	3.00	2.87

Appendix C.2
Oxidation-reduction potential data for multiple grass addition test work to establish whether incremental addition of fresh grass significantly affected the change in pH of the AMD.

Grass Site Identification	Grass Classification	Day 0 Original ORP (mV)	Day 1 After 1st grass addition	Day 3	Day 4 After 2nd grass addition	Day 7	Day 7.5 After 3rd grass addition	Day 8	Day 9	Day 10 1 day after 3rd grass removal
G6 – RD	*Eragrostis Curvula*	489	492	486	490	483	482	476	476	476
G6 – Synthetic	*Eragrostis Curvula*	321	304	254	262	243	154	183	187	193
G13 (2) – RD	*Hyparrhenia hirta*	489	492	488	493	484	483	479	480	480
G13 (2) – Synthetic	*Hyparrhenia hirta*	321	165	188	228	164	138	152	158	164
G15 – RD	*Chrysopogon zizanioides*	489	490	481	485	475	469	463	460	460
G15 – Synthetic	*Chrysopogon zizanioides*	321	111	118	150	101	72	83	109	143
G12 – RD	*Hyparrhenia hirta*	489	491	483	485	474	467	461	458	457
G12 – Synthetic	*Hyparrhenia hirta*	321	161	147	163	133	100	100	108	156
G5 – RD	*Hyparrhenia filipendula*	489		487	491	480	477	471	470	473
G5 – Synthetic	*Hyparrhenia filipendula*	321	193	223	248	195	130	133	196	191
G13 – RD	*Hyparrhenia hirta*	489	491	481	492	481	475	475	473	474
G13 – Synthetic	*Hyparrhenia hirta*	321	154	181	219	131	102	133	146	149
Blank – RD	*Blank – No grass*	489	489	498	509	243	469	533	473	580
Blank – Synthetic	*Blank – No grass*	321	321	311	312	321	477	323	337	353

Appendix C.3
DO data for multiple grass addition test work to establish whether incremental addition of fresh grass significantly affected the change in pH of the AMD.

Grass Site Identification	Grass Classification	Day 0 Original DO (%)	Day 1 After 1st grass addition	Day 3	Day 4 After 2nd grass addition	Day 7	Day 7.5 After 3rd grass addition	Day 8	Day 9	Day 10 1 day after 3rd grass removal
G6 – RD	*Eragrostis Curvula*	28.7	79.8	60.7	67.3	66.4	73.7	55.1	5.31	74.2
G6 – Synthetic	*Eragrostis Curvula*	72.9	78.6	57.8	50	41.5	66.4	15.0	0.83	4.6
G13 (2) – RD	*Hyparrhenia hirta*	28.7	71.8	55.9	67.4	64	76.8	71.5	3.89	12
G13 (2) – Synthetic	*Hyparrhenia hirta*	72.9	72.1	64.5	52.3	61.1	64.2	19.5	0.86	13
G15 – RD	*Chrysopogon zizanioides*	28.7	76.3	62.5	51.6	60.5	64	42.5	3.24	65.8

(*continued on next page*)

Appendix C.3 (*continued*)

Grass Site Identification	Grass Classification	Day 0 Original DO (%)	Day 1 After 1st grass addition	Day 3	Day 4 After 2nd grass addition	Day 7	Day 7.5 After 3rd grass addition	Day 8	Day 9	Day 10 1 day after 3rd grass removal
G15 – Synthetic	*Chrysopogon zizanioides*	72.9	65.1	17.6	15.2	7.7	17.1	12.1	0.35	10.7
G12 – RD	*Hyparrhenia hirta*	28.7	80.7	58.1	66.7	68.3	74	64.1	4.45	72.6
G12 – Synthetic	*Hyparrhenia hirta*	72.9	73.2	40.8	37.1	33.4	50.1	15.8	0.19	12.9
G5 – RD	*Hyparrhenia filipendula*	28.7	76.8	66.9	67.1	62.7	77.6	71.8	4.41	73.7
G5 – Synthetic	*Hyparrhenia filipendula*	72.9	82.5	58.6	55.3	18.5	69.7	21.4	0.51	8.7
G13 – RD	*Hyparrhenia hirta*	28.7	74.9	70.7	67.7	68.7	74.2	65.8	4.93	77.2
G13 – Synthetic	*Hyparrhenia hirta*	72.9	75.8	20.1	62.9	4.5	68.9	10.9	0.61	5.3
Blank – RD	*Blank – No grass*	28.7	28.7	19.2	10.1	10.2	20.5	8.9	2	47.5
Blank – Synthetic	*Blank – No grass*	72.9	72.9	73.2	69.6	64.9	71	64.5	3.85	49.5

Appendix C.4

Hydrogen ion (H^+) data for multiple grass addition test work to establish whether incremental addition of fresh grass significantly affected the change in pH of the AMD.

Grass Site Identification	Grass Classification	Day 0 Original H^+ (mmol/L)	Day 1 After 1st grass addition	Day 3	Day 4 After 2nd grass addition	Day 7	Day 7.5 After 3rd grass addition	Day 8	Day 9	Day 10 1 day after 3rd grass removal
G6 – RD	*Eragrostis Curvula*	10.47	11.22	11.75	6.17	5.89	6.61	6.31	6.31	7.24
G6 – Synthetic	*Eragrostis Curvula*	1.17	0.65	0.60	0.32	0.26	0.13	0.14	0.16	0.18
G13 (2) – RD	*Hyparrhenia hirta*	10.47	10.72	10.23	5.62	5.13	5.13	5.13	5.50	5.89
G13 (2) – Synthetic	*Hyparrhenia hirta*	1.17	0.17	0.12	0.07	0.03	0.01	0.01	0.02	0.02
G15 – RD	*Chrysopogon zizanioides*	10.47	10.72	12.02	5.89	5.01	5.25	5.01	5.25	6.46
G15 – Synthetic	*Chrysopogon zizanioides*	1.17	0.09	0.08	0.05	0.03	0.02	0.03	0.03	0.05
G12 – RD	*Hyparrhenia hirta*	10.47	10.72	10.96	5.62	4.47	5.01	5.25	4.79	5.25
G12 – Synthetic	*Hyparrhenia hirta*	1.17	0.12	0.17	0.08	0.05	0.03	0.04	0.04	0.04
G5 – RD	*Hyparrhenia filipendula*	10.47	10.23	11.75	6.31	5.50	5.25	5.75	6.46	7.08
G5 – Synthetic	*Hyparrhenia filipendula*	1.17	0.44	0.44	0.26	0.17	0.07	0.08	0.09	0.15
G13 – RD	*Hyparrhenia hirta*	10.47	10.23	10.23	5.75	5.50	5.13	5.25	5.25	7.24
G13 – Synthetic	*Hyparrhenia hirta*	1.17	0.21	0.19	0.10	0.03	0.02	0.03	0.02	0.02
Blank – RD	*Blank – No grass*	10.47	10.47	11.48	5.62	5.25	4.90	5.01	5.13	6.03
Blank – Synthetic	*Blank – No grass*	1.17	1.17	1.45	0.76	0.79	0.89	0.91	1.00	1.35

Appendix C.5

Percentage change in hydrogen ion concentration (H+) between days for multiple grass addition testwork.

Grass Site Identification	Grass Classification	% initial change in H^+ (Day 1) After 1st grass addition	% change in H^+ (Day 1–3)	% change in H^+ (Day 3–4) After 2nd grass addition	% change in H^+ (Day 4–7)	% change in H^+ (Day 7–7.5) After 3rd grass addition	% change in H^+ (Day 7.5–8)	% change in H^+ (Day 8–9)	% change in H^+ (Day 9–10) After 3rd grass removal
G6 – RD	*Eragrostis Curvula*	7.2	4.7	−47.5	−4.5	12.2	−4.5	0.0	14.8
G6 – Synthetic	*Eragrostis Curvula*	−45.0	−6.7	−47.5	−16.8	−48.7	4.7	12.2	12.2
G13 (2) – RD	*Hyparrhenia hirta*	2.3	−4.5	−45.0	−8.8	0.0	0.0	7.2	7.2
G13 (2) – Synthetic	*Hyparrhenia hirta*	−85.5	−29.2	−38.3	−56.3	−57.3	−2.3	51.4	2.3
G15 – RD	*Chrysopogon zizanioides*	2.3	12.2	−51.0	−14.9	4.7	−4.5	4.7	23.0
G15 – Synthetic	*Chrysopogon zizanioides*	−92.8	−6.7	−33.9	−36.9	−32.4	14.8	20.2	51.4
G12 – RD	*Hyparrhenia hirta*	2.3	2.3	−48.7	−20.6	12.2	4.7	−8.8	9.6
G12 – Synthetic	*Hyparrhenia hirta*	−89.8	38.0	−51.0	−39.7	−36.9	31.8	−12.9	12.2
G5 – RD	*Hyparrhenia filipendula*	−2.3	14.8	−46.3	−12.9	−4.5	9.6	12.2	9.6
G5 – Synthetic	*Hyparrhenia filipendula*	−62.8	0.0	−39.7	−33.9	−58.3	9.6	7.2	73.8
G13 – RD	*Hyparrhenia hirta*	−2.3	0.0	−43.8	−4.5	−6.7	2.3	0.0	38.0

(*continued on next page*)

Appendix C.5 (*continued*)

Grass Site Identification	Grass Classification	% initial change in H^+ (Day 1) After 1st grass addition	% change in H^+ (Day 1–3)	% change in H^+ (Day 3–4) After 2nd grass addition	% change in H^+ (Day 4–7)	% change in H^+ (Day 7–7.5) After 3rd grass addition	% change in H^+ (Day 7.5–8)	% change in H^+ (Day 8–9)	% change in H^+ (Day 9–10) After 3rd grass removal
G13 – Synthetic	*Hyparrhenia hirta*	−81.8	−12.9	−48.7	−63.7	−42.5	34.9	−18.7	0.0
Blank – RD	*Blank – No grass*	0.0	9.6	−51.0	−6.7	−6.7	2.3	2.3	17.5
Blank – Synthetic	*Blank – No grass*	0.0	23.0	−47.5	4.7	12.2	2.3	9.6	34.9

Appendix C.6

Percentage change in hydrogen ion concentration (H+) compared with initial H+ concentration for multiple grass addition testwork.

Grass Site Identification	Grass Classification	% initial change in H^+ (Day 0–1) 1st grass addition	% change in H^+ (Day 0–3)	% change in H^+ (Day 0–4) After 2nd grass addition	% change in H^+ (Day 0–7)	% change in H^+ (Day 0–7.5) After 3rd grass addition	% change in H^+ (Day 0–8)	% change in H^+ (Day 0–9)	% change in H^+ (Day 0–10) After 3rd grass removal
G6 – RD	*Eragrostis Curvula*	7.2	12.2	−41.1	−43.8	−36.9	−39.7	−39.7	−30.8
G6 – Synthetic	*Eragrostis Curvula*	−45.0	−48.7	−73.1	−77.6	−88.5	−88.0	−86.5	−84.9
G13 (2) – RD	*Hyparrhenia hirta*	2.3	−2.3	−46.3	−51.0	−51.0	−51.0	−47.5	−43.8
G13 (2) – Synthetic	*Hyparrhenia hirta*	−85.5	−89.8	−93.7	−97.2	−98.8	−98.9	−98.3	−98.2
G15 – RD	*Chrysopogon zizanioides*	2.3	14.8	−43.8	−52.1	−49.9	−52.1	−49.9	−38.3
G15 – Synthetic	*Chrysopogon zizanioides*	−92.8	−93.2	−95.5	−97.2	−98.1	−97.8	−97.4	−96.0
G12 – RD	*Hyparrhenia hirta*	2.3	4.7	−46.3	−57.3	−52.1	−49.9	−54.3	−49.9
G12 – Synthetic	*Hyparrhenia hirta*	−89.8	−85.9	−93.1	−95.8	−97.4	−96.5	−97.0	−96.6
G5 – RD	*Hyparrhenia filipendula*	−2.3	12.2	−39.7	−47.5	−49.9	−45.0	−38.3	−32.4
G5 – Synthetic	*Hyparrhenia filipendula*	−62.8	−62.8	−77.6	−85.2	−93.8	−93.2	−92.8	−87.4
G13 – RD	*Hyparrhenia hirta*	−2.3	−2.3	−45.0	−47.5	−51.0	−49.9	−49.9	−30.8
G13 – Synthetic	*Hyparrhenia hirta*	−81.8	−84.2	−91.9	−97.0	−98.3	−97.7	−98.1	−98.1
Blank – RD	*Blank – No grass*	0.0	9.6	−46.3	−49.9	−53.2	−52.1	−51.0	−42.5
Blank – Synthetic	*Blank – No grass*	0.0	23.0	−35.4	−32.4	−24.1	−22.4	−14.9	14.8

Appendix D. Appendix

Appendix D.1

Elemental concentration data of grass samples.

Units	g/t												
Element	Na	Mg	K	Ca	P	S	B	Al	Mn	Co	Cu	Zn	Pb
G13 - Synthetic - Original	368	2011	10,877	4318	1915	4839	3.97	1187	296	1.16	12.1	72.9	2.19
Add 1	7015	171	786	568	1255	10,053	10.1	736	59.7	0.82	8.07	22.5	1.77
Add 2	10,608	396	2101	1338	1367	15,026	1.28	973	108	0.93	9.51	31.3	1.70
Add 3	8084	466	2329	2093	1456	11,529	1.51	837	126	1.05	11.7	42.7	2.97
G13 (2) - Synthetic - Original	408	3510	13,601	6054	2876	6922	8.29	7894	465	4.71	26.4	78.6	6.06
Add 1	7050	237	576	697	1345	9933	1.82	4147	90.2	1.79	12.1	20.1	2.86
Add 2	11,954	583	1682	1777	1308	18,095	10.8	4040	164	2.15	9.84	30.9	3.02
Add 3	7611	555	1532	2358	1470	11,839	2.23	3370	173	2.19	11.1	36.8	3.99
G6 - Synthetic - Original	7.87	489	4897	2417	592	1065	2.07	240	40.7	0.36	6.77	20.4	21.17
Add 1	3868	281	1952	476	448	5292	0.81	498	28.6	0.45	8.30	9.85	0.92
Add 2	3962	331	1373	915	508	5388	10.4	257	22.4	0.38	7.18	25.3	2.34
Add 3	3172	67.5	562	351	301	4497	0.50	274	16.2	0.29	7.88	7.25	1.36
G5 - Synthetic - Original	5.54	1106	11,052	2201	1272	1530	3.71	384	92.0	0.54	3.84	21.5	1.10
Add 1	4224	681	614	3904	1811	6558	13.2	1086	120	2.06	23.7	194	20.1
Add 2	8328	315	1903	666	695	13,062	1.19	499	59.8	0.85	4.78	12.2	2.30
Add 3	5865	213	1884	552	604	7947	1.21	318	33.6	0.42	6.97	9.73	0.78
G15 - Synthetic - Original	221 *±11*	3234 *±117*	11,935 *±554*	6734 *±283*	1817 *±198*	1814 *±45.1*	6.80 *±0.21*	355 *±64.6*	45.6 *±3.09*	0.42 *±0.13*	10.8 *±1.13*	79.3 *±3.41*	4.88 *±0.73*

(*continued on next page*)

Appendix D.1 (*continued*)

Units	g/t												
Element	Na	Mg	K	Ca	P	S	B	Al	Mn	Co	Cu	Zn	Pb
Add 1	5362 ± *265*	261 ± *1.66*	843 ± *3.88*	2416 ± *51*	1173 ± *24.5*	7557 ± *89.3*	1.20 ± *0.007*	385 ± *3.41*	24.7 ± *0.14*	0.41 ± *0.00*	7.67 ± *0.16*	13.3 ± *0.03*	1.18 ± *0.06*
Add 2	4736	389	1273	3386	1141	6456	11.1	298	22.5	0.28	5.66	14.3	1.45
Add 3	4099	476	1600	3728	888	5844	8.34	309	21.9	0.28	8.51	14.3	0.02

Appendix D.1
ICP analysis data of grass samples - continued.

Units	g/t												
Element	Na	Mg	K	Ca	P	S	B	Al	Mn	Co	Cu	Zn	Pb
G12 - Synthetic - Original	42.7	3900	27,542	4360	2970	4022	7.82	3156	181	1.94	12.8	57.0	3.14
Add 1	6732	672	2423	1148	2240	9088	2.73	3671	73.5	1.93	10.9	42.8	3.15
Add 2	9810	1143	5312	2969	2403	14,155	13.2	3987	114	2.21	13.4	36.0	3.35
Add 3	4896	961	4285	4019	2110	6750	3.47	3298	97.5	1.97	14.6	40.1	3.65
G12 - RD - Original	42.7	3900	27,542	4360	2970	4022	7.82	3156	181	1.94	12.8	57.0	3.14
Add 1	128	1208	2145	1608	998	14,749	2.07	3859	218	4.42	14.3	39.0	6.32
Add 2	157	1333	3164	1615	1339	14,842	2.73	5088	222	4.69	16.1	42.8	4.16
Add 3	73.9	958	2572	1251	1958	9388	2.73	3654	144	3.30	14.9	29.3	2.68
CRM - Tea Leaves CRM	6.76 ±*0.00*	2261 ±*48.9*	15,729 ±*88.7*	5711 ±*28.7*	2418 ± *8.50*	2606 ±*12.7*	28.1 ± *0.26*	2131 ±*15.6*	1505 ±*17.4*	0.30 ±*0.002*	21.3 ±*1.45*	32.2 ±*0.30*	1.68 ±*0.13*
CRM – True values	24.7 ± *3.2*	2240 ± *170*	17,000 ± *1200*	5820 ± *520*	_	2470 ± *250 %*	_	2290 ± *250*	1570 ± *110*	0.387 ± *0.042*	20.4 ± *1.5*	34.7 ± *2.7*	1.78 ± *0.024*

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