Investigating the use of polyhalite as a potassium fertiliser in rice plants

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Abstract

Potassium (K) is essential for plant growth and health, featuring in a vast array of activities within the plant. Deficiencies in K nutrition cause serious issues regarding plant growth and crop yield. Polyhalite ($K_2SO_4.MgSO_4.2CaSO_4.2H_2O$) is a hydrated evaporate mineral which can be used directly as a K fertiliser. However, there has been limited work on the suitability and efficacy of polyhalite compared to traditionally used K fertilisers such as potassium chloride (KCl) and potassium sulphate (K_2SO_4). The aims of this thesis were to explore the efficacy of polyhalite as a K fertiliser compared to KCl and K_2SO_4 and evaluate its effect on rice growth traits and nutrient content in both balanced and unbalanced fertiliser regimes. No differences in rice biomass were discovered between different K fertiliser regimes. However nutrient analyses showed inconsistent results to the different K fertilisers. A range of rice cultivars was then assessed for their response to different K fertiliser regimes. No significant variation between individual cultivars was observed in response to different K fertiliser sources with respect to biomass or nutrient content. Additionally, grouping cultivars by potassium use efficiency provided few changes in nutrient content responses. Finally, microbial communities in the plant rhizosphere were characterised for taxonomic diversity to determine if and how these properties are affected by different K fertilisers. In nutritionally balanced fertiliser regimes, the bacterial populations of the rice plant rhizosphere were not influenced by treatment although harvesting timepoint did affect their composition. Conversely, comparison of unbalanced K regimes revealed differences in bacterial species composition, but little change was observed in the total number or distribution of species of the bacterial populations. The findings of this work can be used to inform similar investigations using polyhalite as a K fertiliser as well as future work investigating K fertiliser effects on microbial populations.

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Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Abbreviations

AE: Agronomic Efficiency

ASV: Amplicon sequencing variants

CEC: Cation exchange capacity

CRF: Controlled release fertiliser

DMNP: Dry Matter Nutrient Productivity

DMUE: Dry Matter Utilisation Efficiency

FUE: Fertiliser Use Efficiency

GWAS: Genome wide association study

ICP – OES: Inductively coupled plasma – optical emission spectrophotometry

IE: Internal Efficiency

KUE: Potassium use efficiency

KUpE: ability of the plant roots to utilise the K within the soil

KUtE: ability of the plant to convert the K it has procured into yield

MOP: Muriate of potash

NMDS: Non-metric multidimensional scaling

PCA: Principal component analysis

PE: Physiological Efficiency

PFB: Partial Nutrient Balance

PNB: Partial Nutrient Balance

PSI: Photosystem I

PSII: Photosystem II

RE: Apparent Recovery Efficiency

RGR: Relative growth weight

RUBISCO: Ribulose-1,5-bisphosphate carboxylase-oxygenase

SRF: Slow-release fertiliser

SOP: Sulphate of Potash

SUE: Shoot Nutrient Productivity

TGW: Thousand grain weight

T-RFLP: Terminal restriction fragment length polymorphism

Chapter 1: Introduction

1.1 The Demands on Global Crop Production

1.1.1 Agriculture and the global human population

The proliferation of the world's human population has led to increased pressure being placed on the global agricultural system. Many populations have increasing prosperity leading to changes in diets, including higher meat consumption. The higher proportion of a more energy intensive food source places further demands on farming systems (Davis et al., 2016). Alongside this, extensive issues can be observed in food distribution, with estimates of food loss and wastage at all stages of production suggesting that approximately 30% of food produced is not consumed (Gustavsson et al., 2011). Nutritional concerns are also growing as obesity and malnourishment are major problems across the globe. Lack of access to diverse foodstuffs is a problem for the majority of under-malnourished individual. Paradoxically, many individuals classed as morbidly obese also have micronutrient deficiencies due to poor dietary options (Kaidar-Person et al., 2008).

The introduction of intensive farming methods during the Green Revolution, led to large increases in crop yields worldwide, however they also raised many problems due to the reliance on chemical based pesticides, herbicides and fertilisers combined with the dependence on machinery and the use of fossil fuels (Pellegrini & Fernández, 2018). An estimated 40-60% of current crop production is aided by fertiliser applications (Johnston & Bruulsema, 2014). Our current reliance on inorganic fertilisers to sustain a sufficient level of food production is a difficult issue to overcome, however whilst we search for more sustainable fertilisation methods it is also vital that we investigate new sources of inorganic fertilisers to fill any shortfalls that may be seen from diminishing supplies of current fertiliser sources.

1.1.2 Nutrients required for plant growth

The nutrients nitrogen (N), phosphorus (P) and potassium (K) are generally recognised as being the most important for plant growth, however sulphur (S), calcium (Ca) and magnesium (Mg) are also classed as macronutrients due to the large quantities plants require for effective growth. Furthermore, plants also require a number of micronutrients which are

needed in much smaller quantities and include Boron (B), Copper (Cu), Chlorine (Cl), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (N) and Zinc (Zn) (fig 1.1).

The act of harvesting crops inevitably removes nutrients from the field system and must therefore by replaced for future crop growth. Application of additional nutrients to the soil is there essential for crop growth and optimising crop yields. It is important to understand the role of each nutrient within the plant. Recognising nutrient deficiencies and toxicities as well as understanding nutrient uptake mechanisms and soil chemistry interactions all contribute to a better understanding of how optimal conditions can be maintained for plant growth.

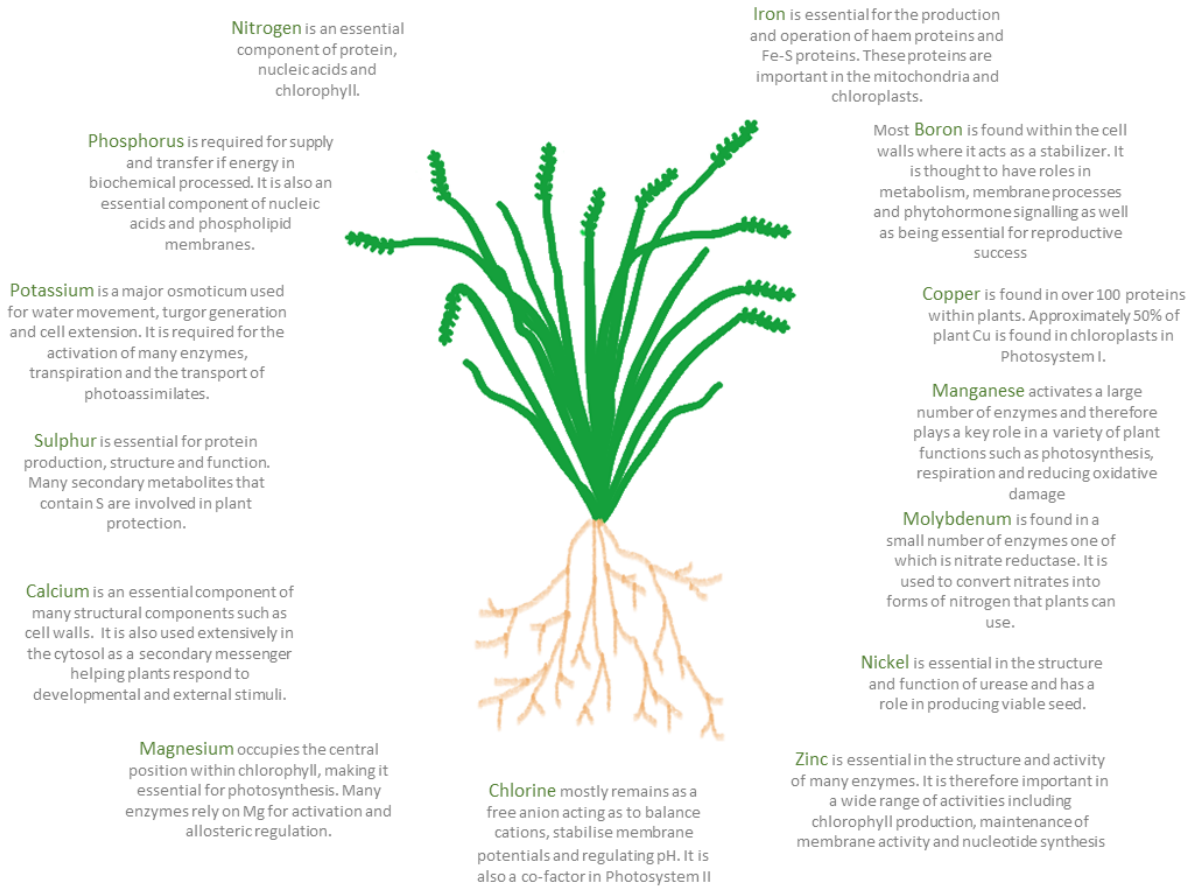


Figure 1.1 – Roles of essential plant nutrients

1.2 The role of macronutrients in plant development and health

1.2.1 Nitrogen

1.2.1.1 Role of nutrient in plant development

The macronutrients generally recognised as the most important for plant growth are N, P and K with N being required in the highest quantities with total plant dry matter consisting of 1-5% N. Nitrogen is an integral component of proteins and nucleic acids as well as chlorophyll, co-enzymes, phytohormones and secondary metabolites (Marschner, 2012). Plants can utilise a variety of different N forms, including ammonium ions (NH_4^+) and nitrate ions (NO_3^-) as well as being able to access N through symbiotic relationships with soil microorganisms (Lea and Azevado 2007). Once taken up, nitrate is converted into ammonia within the plant in two steps using the enzymes nitrate reductase to convert nitrate to nitrite and nitrite reductase to convert nitrite to ammonia (Hirel et al 2011). The use of the enzymes glutamate synthase and glutamine synthetase, allow ammonia to be incorporated into the amino acids glutamate and glutamine (Hirel et al 2011). These two amino acids then act as amino acid group donors to all other N-containing molecules, notably nucleotides for RNA and DNA synthesis, proteins, as well as low molecular weight compounds which are used to store N in periods of high N availability (Marschner, 2012). Remobilization of N occurs at senescence and grain filling stages providing seeds with N reserves for growth and development (McAllister et al 2012).

1.2.1.2 Symptoms of deficiency

Plants with N deficiencies tend to be stunted, with small thin leaves which are often pale green to yellow in colour. Older leaves usually present symptoms of chlorosis first as N is remobilized to younger leaves (Barker & Pilbeam, 2007). With extended periods of limited N provision, leaf nucleic acids and proteins are broken down which often leads to leaf senescence. A decrease in photosynthetic activity due to reduced ribulose-1,5-bisphosphate carboxylase-oxygenase (RUBISCO) concentrations inhibits plant growth and reduces plant yields (Marschner, 2012).

1.2.2 Phosphorus

1.2.2.1 Role of nutrient in plant development

Phosphorus remains in its oxidised form within the plant as either soluble orthophosphate or as pyrophosphate (Maathuis, 2009). Excess P is stored in the vacuole which can be used if P sources decline (Veneklaas et al., 2012) but also allows for careful regulation of many metabolic pathways (Marschner, 2012). Phosphorus is required for the supply and transfer of energy in all biochemical processes within the plant, as it is an essential component of the compounds adenosine di- and triphosphate (ADP and ATP). It is also an important constituent of nucleic acids, allowing the bridging of nucleoside units to form DNA and RNA molecules (Marschner, 2012). Phospholipid membranes are another structure which contain P, with an orthophosphate molecule linking the lipophilic and hydrophilic regions of each phospholipid (Maathuis, 2009).

The reversible action of protein phosphorylation is significant in modulating protein activity (Maathuis, 2009) and is thought to be a vital part of signal transduction (Marschner, 2012).

Phosphorus is stored in seeds as phytate which allows for embryo development, germination and early seedling growth (Maathuis, 2009). Developing seeds can use this P source for synthesis of membrane lipids and nucleic acids (Marschner, 2012) and can provide improved access to resources essential for growth due to improvements to seedling vigour (Veneklaas et al., 2012).

1.2.2.2 Symptoms of deficiency

Phosphorus deficiency decreases leaf expansion and number of leaves produced, however visual symptoms on the foliage are not frequently observed. Deficient plants may have darker green leaves, as chlorophyll formation is less strongly affected than leaf expansion in P limiting conditions (Barker & Pilbeam, 2007; Marschner, 2012). Species and cultivars which produce anthocyanins may have leaves which turn red-purple in colour (de Datta, 1981).

Due to the high mobility of P within the plant, it is easily translocated from older leaves to developing leaves and therefore symptoms appear in older leaves first (Barker & Pilbeam, 2007). The shoot/root ratio can also be reduced as root growth is less affected than shoot growth (Marschner, 2012).

1.2.3 Potassium

1.2.3.1 Role of nutrient in plant development

After N, K is the most abundant nutrient within plants (Roy et al., 2006). Whilst both N and P are utilised by the plant in structural forms, K remains as an ion, allowing it high mobility within the plant. Cellular levels of K are maintained at high concentrations (Leigh & Wyn Jones, 1984), however there are large differences between different compartments of the cell with concentrations within the cytosol reaching up to 80 mM whereas the vacuole can reach concentrations of 120 mM (Walker et al., 1996).

Potassium cations are an extremely important osmoticum within plants (Zörb et al., 2014). Within the vacuole, K is important for lowering osmotic potential which enables turgor generation and cell extension (Walker et al., 1996) as well as balancing charges from accompanying anions. Furthermore K is often stored within the vacuole, which typically occurs when K is available in excess (Ragel et al., 2019). Many plant movements rely on K, for example in stomatal functioning where influx and efflux of K into the guard cells surrounding the stomata aperture results in uptake and loss of H_2O from adjacent cells. This subsequently leads to the regulation of stomatal opening and closing, helping plants to control transpiration (Marschner, 2012).

Within the cytosol and chloroplasts, K concentrations remain fairly constant, which facilitates a stable pH between 7-8 within these compartments, optimal for many enzymatic reactions (Marschner, 2012). Approximately 60 enzymes have been identified as requiring K for their activity or stimulation. Potassium allows conformational changes to enzymes which increase their rate of reaction and sometimes also their affinity for the substrate (Marschner, 2012). Many of these enzymes are involved in sugar and N metabolism (Amtmann et al., 2008) however they are also present in processes such as glycolysis, protein synthesis, nucleic acid and nucleotide metabolism as well as amino acid metabolism (Evans & Sorger, 1966; Marschner, 2012).

Potassium plays an important role in maintaining the optimal conditions for photosynthetic activity. Transport of photoassimilates is also facilitated by K through its contribution to the osmotic potential of the sieve tubes, enabling transport from source to sink

organs, as well as maintenance of a high pH in the sieve tubes required for sucrose loading (Marschner, 2012).

In addition K is essential for plant health, playing a part in a vast array of activities, including protection against frost damage (Grewal & Singh, 1980), protection against disease (Cakmak, 2005) and protection against drought stress (Sardans & Peñuelas, 2015).

1.2.3.2 Symptoms of deficiency

Deficiencies in K nutrition cause inhibition of plant growth due to its extensive role within the plant. Due to the rapid redistribution of K between plant tissues it is not always easy to detect K deficiency; however, it manifests itself in reduced growth rates and in more serious cases as chlorosis and necrosis of the leaves (Römheld & Kirkby, 2010). Total root growth is affected by reduced K availability, observed by the reduced number and length of lateral roots when plants are presented with low K medium (Drew, 1975).

Potassium plays an important role in maintaining photosynthetic levels and thus K-deficient plants have severe reductions in photosynthetic activity, with reduced RUBSICO activity (Weng et al., 2007) and are limited in their ability to effectively use photosynthates (Cakmak, 2005; Kanai et al., 2011). The reduction in photosynthetic activity leads to a build-up of electrons which in turn stimulates reactive oxygen series (ROS) production which is exacerbated in high-light intensities and can cause chlorosis and necrosis to occur more rapidly (Cakmak, 2005). Additionally, reduced photosynthetic activity leads to an accumulation of sugars in the leaves, due to reduced phloem loading. This increase in soluble sugars as well as organic acids can lead to increased susceptibility to pathogens as these compounds provide a desirable food source. Additionally impairments to stomatal movements can reduce the effectiveness of plant defences as many pathogens use these apertures to access the interior of the plant (Zörb et al., 2014).

Low temperature stresses are exacerbated in K-limiting conditions as photosynthetic electron transport, stomatal conductance, RUBISCO activity and CO_2 fixation are all factors impaired both in low temperatures and K deficient conditions. Similarly drought stresses are also intensified in K deficient plants due to the role of K as an osmoticum (Cakmak, 2005).

1.2.4 Calcium

1.2.4.1 Role of nutrient in plant development

The majority of plant Ca is utilised in structural or regulatory components of macromolecules, with a high proportion being found in the cell walls. Calcium binds to the negative charges in pectin, allowing inter-polymer bridges to form in the middle lamella of cell walls. Higher concentrations of Ca within the pectin formed matrix allow higher levels of cross-linking between polymer chains, increasing the load bearing strength of the cell walls and plant tissue. These increases in cell wall strength improve plant vigour and seed and fruit development (Marschner, 2012; Barker & Pilbeam, 2007; Hirschi, 2004). Calcium binding to phospholipids is also thought to stabilise lipid bilayers, providing structural integrity to cellular membranes and regulating the permeability of the plasma membrane (Hepler, 2005).

Calcium is also used extensively as a secondary messenger (Kudla et al., 2010). Minute fluctuations in cytosolic Ca ion concentrations can determine how plants respond to a variety of developmental and external cues (Hirschi, 2004). The primary target of Ca are sensor proteins such as calmodulins, calcineurin-b like proteins, Ca-dependent protein kinases and annexins. By binding to these proteins, Ca causes alterations to the protein's conformation or enzymatic properties therefore causing changes to solute transport, metabolism, cell morphology and gene expression (Marschner, 2012). As Ca regulates multiple signalling pathways it is thought that there are different Ca signatures with specific amplification, duration and oscillations of Ca ions which encode distinctive instructions (Seybold et al., 2014).

Cytosolic Ca ion levels are maintained at an extremely low concentration (~100 nM) by membrane bound Ca dependent ATP fuelled pumps (Hetherington & Brownlee, 2004). When the Ca supply is increased, excess Ca ions are stored in the vacuole. As well as reducing cross talk between signals, low cytosolic Ca concentrations also prevent unregulated activation of Ca dependent enzymes. Competition with Mg ions for enzyme binding sites is also an issue with raised cytosolic Ca concentrations. For example concentrations of just 1µM Ca can severely inhibit the activity of fructose 1,6 – biphosphatase, even when Mg concentrations are much higher. (Marschner, 2012).

Ca signalling plays an important role in the initial phases of symbioses such as those of legumes and nitrogen fixing rhizobia or the formation of arbuscular mycorrhizae (Oldroyd & Downie, 2006). However, bacterial and fungal pathogens also induce Ca signals in the cytosol and nucleus in response to different elicitors such as flagellins and oligogalacturonides (Kudla et al., 2010).

1.2.4.2 Symptoms of deficiency

Calcium deficiency is uncommon due to its abundance in the soil however it can occur in highly weathered tropical soils and on strongly acidic, saline and sodic soils or through unbalanced fertiliser applications (White & Holland, 2018; Barker & Pilbeam, 2007). As Ca cannot be redistributed from older leaves, Ca disorders frequently occur in horticultural produce, in young leaves and fruit where high demands of Ca cannot be met.

Symptoms of Ca deficiency are initially as chlorosis followed by necrosis and can lead to plant tissue collapse through cell walls disintegrating. Calcium limitations can also lead to premature pollen release and failure to set seed. These problems lead to characteristic disorders such as blossom end rot in tomatoes, blackheart in celery and bitter pit in apples (White & Holland, 2018). Increased susceptibility to bacterial infections, fruit rotting and other postharvest problems are also often seen when Ca is a limited resource (Hirschi, 2004).

1.2.5 Magnesium

1.2.5.1 Role of nutrient in plant development

Magnesium plays a pivotal role in photosynthesis due to its central atomic position within the chlorophyll molecule. It is also integral to the production of chlorophyll structures as magnesium chelatase catalyses the first steps of chlorophyll biosynthesis (Cakmak & Kirkby, 2008). It is also involved in grana stacking with Mg deficiencies resulting in disorganised thylakoid membranes (Verbruggen & Hermans, 2013).

The activation of many enzymes including RUBISCO (Cakmak & Kirkby, 2008), RNA polymerases, ATPases and protein kinases (Shaul, 2002) rely on Mg as a co-factor and allosteric modulator for over 300 different enzymes (Verbruggen & Hermans, 2013). Protein production is also impaired in Mg deficient plants, as Mg has an important function in aggregating and stabilising the subunits of ribosomes (Marschner, 2012; Barker & Pilbeam, 2007). Vacuole

concentrations of Mg are high, enabling it to aid in balancing the charge of anions and maintenance of cell turgor (Maathuis, 2009). High Mg contents have also been seen to alleviate aluminium (Al) stress, with rice plants overexpressing the Mg transporter gene *OsMGT1* showing resistance to Al toxicity (Chen et al., 2012).

Phloem loading and the transport of photo-assimilates from source to sink organs is also thought to be facilitated by Mg (Cakmak, 2013; Tanoi & Kobayashi, 2015; Cakmak et al., 1994). Phloem loading of sucrose is an active process requiring an H^+-ATPase to establish a proton gradient in the sieve tube cells (Cakmak et al., 1994). A decrease in ATP concentrations at phloem loading sites due to a depletion in available Mg has been suggested to be a reason for the inhibition of sucrose loading in Mg deficient plants (Cakmak & Kirkby, 2008).

1.2.5.2 Symptoms of deficiency

Impairments in sugar partitioning leads to the accumulation of photosynthates and starches in young source leaves of Mg deficient plants causing alterations to photosynthetic C metabolism and restricting CO_2 fixation (Tanoi & Kobayashi, 2015). These restrictions to photosynthesis can generate a build-up of non-utilised electrons which may then be channelled into ROS production and consequently damage to chloroplast components (Cakmak & Kirkby, 2008). The photooxidative damage of chlorophyll and inhibition of chlorophyll biosynthesis due to low Mg availability leads to interveinal chlorosis, however this is a late symptom of Mg deficiency, by which time crop yield losses will already have been incurred. High light intensities exacerbate these chlorosis symptoms (Gransee & Führs, 2013). Reduced root growth has been seen in response to reduced Mg availability in some plant species, however not all seem to respond in this way (Gransee & Führs, 2013; Hermans & Verbruggen, 2005; Hariadi & Shabala, 2004). The circadian clock is also disrupted when Mg is deficient as alterations are seen in the central oscillator within the clock pathway (Hermans et al., 2010).

1.2.6 Sulphur

1.2.6.1 Role of nutrient in plant development

The essential amino acids, methionine and cysteine contain S, making it an indispensable component for protein synthesis. Disulfide bridges between cysteine residues

play an important part in protein production, structure and function (Marschner, 2012). In addition, S is utilised by several coenzymes required for protein synthesis (Dobermann & Fairhurst, 2000). Many S containing secondary metabolites play an important role in a plant's protective mechanisms, for example glucosinolates in the Brassicaceae family and allicin in the Alliaceae family, which, as well as helping to deter pests and protest against diseases, are also beneficial to human health (Prasad et al., 2018). Further secondary metabolites include phytosulfokines, which control cell growth, somatic embryogenesis and pathogen defence (Koprivova & Kopriva, 2016) and sulfolipids which are found in the membranes of chloroplast thylakoids (Maathuis, 2009). Other S containing secondary metabolites contribute to crop quality traits including, baking and milling qualities in wheat, increased chlorophyll content in red clover and increased vitamin A content in alfalfa, leading to improved forage quality (Ceccotti, 1996).

1.2.6.2 Symptoms of deficiency

Sulphur deficiencies strongly resemble the symptoms of nitrogen deficiency, making it difficult to distinguish between the two simply with visual indicators (Dobermann & Fairhurst, 2000). Shoot growth is decreased, and fruit maturation can be delayed. Whilst the root is less affected in most plants, root nodulation in legumes can be impaired (Ceccotti, 1996). Foliar chlorophyll content is often dramatically decreased under sulphur limiting conditions. Protein synthesis is also inhibited which can lead to leaf chlorosis (Marschner, 2012) and sub-optimal N utilisation therefore lowering a plant's efficient use of applied N fertilisers (Ceccotti, 1996). As S is less mobile than N, chlorosis tends to be more severe in young leaves (Scherer, 2001). Shortages in S can also limit the effectiveness of the plant's response to Fe and reduce the plant's Fe use efficiency (Astolfi et al., 2020).

1.3 The role of micronutrients in plant development and health

1.3.1 Boron

1.3.1.1 Role of nutrient in plant development

The role of B in plant nutrition is not well understood and separating the primary and secondary effects of B deficiency is difficult. Therefore, B has been implicated as having roles in a range of processes in metabolism, membrane processes and phytohormone signalling.

Approximately 90 % of the total B content of plants is localised within the cell walls (Blevins & Lukaszewski, 1998). Whilst B does not appear to be involved in cell wall synthesis it does help to stabilise walls as it acts to cross-link subunits of pectin polysaccharide rhamnogalacturonan II by their apiosyl residues within cell wall structures (Dannel et al., 2002; Matthes et al., 2020). Variability in plant B requirements appear to be correlated with the cell wall pectin content (Dannel et al., 2002).

Boron is also essential for reproductive success; however, it is still unclear whether reproductive tissues demand higher levels of B than vegetative tissues or whether their sensitivity to B deficiency is due to reduced delivery of B to these systems. Pollen tube growth and anther development are particularly sensitive to limiting B conditions (Brown et al., 2002). Whilst pollen grains are usually low in B, the style, stigma and ovaries tend to be high in B and a continuous and plentiful supply of B is required for pollen tube growth (Blevins & Lukaszewski, 1998).

1.3.1.2 Symptoms of deficiency

Rapid inhibition of both shoot and root growth are early symptoms of B deficiency due to a reduction in cell elongation and cell division (Matthes et al., 2020). In some species, growth inhibition is followed by tissue death whereas in other species this does not occur although mild chlorosis may present as a secondary symptom (Marschner, 2012). Due to the importance of B in cell wall structure, B deficiency causes strong alterations to cell walls which can be seen at the macroscopic and microscopic levels. At the microscopic level, cell walls become abnormally thick and brittle, have structural deformations, altered mechanical properties and don't expand normally. Macroscopic changes show in brittle leaves and in symptoms such as water-soaked areas, tipburn and brown- or blackheart that occur in vegetable crops (Marschner, 2012; Brown et al., 2002).

Fruit and seed yields are often severely affected by limited B supply. Plants under moderate to severe B deficiency fail to produce functional flowers and may produce no seeds. Often B deficiency will not be apparent from vegetative growth, however seed yield can be dramatically decreased and often leads to reduced seed viability (Barker & Pilbeam, 2007; Marschner, 2012).

1.3.2 Copper

1.3.2.1 Role of nutrient in plant development

Within plants, Cu is found in over 100 different proteins. The ability to change between oxidation states means it is often utilised in reduction and oxidation reactions required for single electron transfer reactions in Cu- containing proteins and enzymes (Barker & Pilbeam, 2007). Copper is an important metal in many oxidase enzymes, attributable to Cu's high affinity to oxygen (Hänsch & Mendel, 2009). Located on the mitochondrial inner membrane, cytochrome c oxidase is an example of a Cu containing oxidase and is the final oxidase in the mitochondrial electron transport chain (Marschner, 2012). Approximately 50 % of the Cu in plants is found in chloroplasts, associated with plastocyanin, a component of the electron transport chain of Photosystem I (PSI). Another Cu cofactor interacts with ethylene binding domains to allow high affinity ethylene binding activities (Rodríguez et al., 1999).

At high concentrations, Cu is toxic to plants causing oxidative damage to plant macromolecules such as DNA and proteins, therefore concentrations of free Cu within the cytoplasm are kept extremely low. To regulate intracellular Cu levels and facilitate its movement, plants use metallochaperones and metallothioneins. Metallochaperones are soluble, intracellular receptors which guide Cu to its target destination and can also aid in the insertion of Cu into the target protein whereas metallothioneins are scavenging proteins that bind the majority of Cu after uptake to prevent it causing oxidative damage (Hänsch & Mendel, 2009).

1.3.2.2 Symptoms of deficiency

Characteristic symptoms of Cu deficiency include; stunted growth, distortion of young leaves, chlorosis and necrosis spotting and leaf bleaching (Marschner, 2012). Male sterility is also a common problem in Cu deficient plants as pollen grains have reduced viability. The role of Cu within many enzymes leads to reduced activity of these enzymes in Cu deficient plants which in turn decreases activities including photosynthesis, C and N metabolism and oxidative stress protection (Barker & Pilbeam, 2007).

Lignification is also affected by limitations in Cu availability, which can lead to distortion in growth morphology and increased susceptibility to lodging in cereal crops

(Marschner, 2012). Another typical symptom of Cu deficiency is wilting in young leaves despite adequate water supply which is thought either to be the consequence of inadequate lignification of xylem vessels reducing water or to structural flaws in cell walls (Rehman et al., 2019; Marschner, 2012).

1.3.3 Chlorine

1.3.3.1 Role of nutrient in plant development

Whilst Cl is classed as a micronutrient it is often present within plants at similar concentrations to that of macronutrients, however the minimum requirement for plant growth is about 10-100 times lower. Chlorine is a co-factor of the Mn-containing complex in the water splitting reaction of Photosystem II (PSII) (Kusunoki, 2007), however it mostly remains as a free anion within the plant and therefore can act as a counter anion, helping to stabilize membrane potentials and regulate pH (Marschner, 2012). Accumulations of Cl in plant cells increases turgor pressure and tissue hydration and it counterbalances K cations in cell expansion and elongation, as well as regulation of the stomatal aperture (Barker & Pilbeam, 2007; Kirkby & Romheld, 2004).

1.3.3.2 Symptoms of deficiency

Symptoms of chlorine deficiency are rarely seen, especially in field settings. When deficiency does occur, it manifests as curling of the youngest leaves, leading to leaf shrivelling and eventually necrosis. Root become stubby through swelling near the apex (Marschner, 2012).

1.3.3.3 Symptoms of toxicity

Plant responses to Cl are very variable and many species and cultivars are sensitive to high levels of Cl. These sensitivities are particularly problematic in many important vegetable and fruit crops and can constrain crop production (White & Broadley, 2001). Differences between cultivar sensitivities are usually related to their ability to restrict Cl transport from root to shoot. Excess chlorine can cause leaf margin curling, leaf necrosis and leaf drop. In severe conditions, the terminal axis and small branches may die off (Barker & Pilbeam, 2007).

1.3.4 Iron

1.3.4.1 Role of nutrient in plant development

Iron is fundamental in the operation and biosynthesis of two major groups of proteins; the haem proteins and the Fe-S proteins (Barker & Pilbeam, 2007). Cytochromes are one of the most well-known group of haem proteins, acting as components of the redox systems in mitochondria and chloroplasts as well as the redox chain of nitrate reductase. Other haem proteins include catalase, superoxide dismutase and peroxidase; antioxidative enzymes which help protect the plant from ROS (Tripathi et al., 2018).

Chloroplasts are particularly rich in Fe-S proteins including the protein complex PSI, ferredoxins as well as other metabolic enzymes. Ferredoxins are mediators in electron transfer, working in electron chains in a number of metabolic reactions. In the thylakoid membrane, approximately 20 Fe atoms are required in the electron transport chain (Marschner, 2012; Schmidt et al., 2020).

1.3.4.2 Symptoms of deficiency

Iron deficiency frequently presents symptoms of interveinal chlorosis, with veins remaining green whilst the laminae turn yellow. In cereal crops this appears as alternating yellow and green stripes across the leaf. Deficiency causes inhibition of chloroplast development leading to changes in the chloroplast structure as they are smaller and the thylakoid grana are absent in severely-limiting conditions. As Fe is often contained in chloroplasts in older leaves it is not readily translocated to younger leaves, which therefore show more pronounced symptoms (Barker & Pilbeam, 2007).

In Fe limiting conditions, plant roots of species which use Strategy I for Fe uptake, which includes all plants except the cereals and grasses, undergo morphological and physiological changes. These changes include inhibition of root elongation and increased density of root hairs and number of lateral roots (Marschner, 2012; Connorton et al., 2017).

1.3.4.3 Symptoms of toxicity

Iron toxicity tends to only be a problem in rice crops and is one of the most important yield-limiting factors in wetland rice production. Symptoms include small reddish-brown spots on the leaves and in acute cases the whole leaf may turn brown with older leaves prematurely

dying (Barker & Pilbeam, 2007; Marschner, 2012). Iron related toxicity damage is caused by Fe reacting with hydrogen peroxide and thus the formation of ROS (Schmidt et al., 2020).

1.3.5 Manganese

1.3.5.1 Role of nutrient in plant development

A relatively large number of enzymes have been identified as being activated by Mn, however only 35 have been identified as requiring Mn as a co-factor and an even smaller number actually contain Mn. These three enzymes are: oxalate oxidase which helps defend against pathogens by destroying fungal toxins as well as generating H_2O_2 which is important in cross-linking steps in lignification, superoxide dismutase and the oxygen-evolving complex in photosystem II (PSII).

Superoxide dismutase scavenges ROS, reducing oxidative damage to cells by catalysing the conversion of superoxide anion free radicals (O_2^-) into molecular oxygen and hydrogen peroxide. There are a variety of isoenzymes of superoxide dismutase which are accompanied by different metal ions, either Fe, Mn, Cu or Zn. The different metal containing isoenzymes can be found in different internal compartments of the cell with Mn superoxide dismutase mainly being located in the mitochondria and peroxisomes (Marschner, 2012).

The unique chemistry of Mn, allowing it to cycle through many redox states makes it an ideal element for the PSII system which requires the Mn_4Ca catalytic cluster to cycle through 5 oxidation states to allow for both the provision of electrons for the photosynthetic electron transport chain as well as for water oxidation (Schmidt & Husted, 2019; Marschner, 2012).

1.3.5.2 Symptoms of deficiency

Typical foliar phenotypes are only seen when a plant's growth rate has been significantly reduced. At this point diffuse interveinal chlorosis can be seen on young expanded leaves and necrotic spots or streaks can also be observed (Schmidt & Husted, 2019).

The role of Mn in PSII means even moderate limitations in Mn availability effect the operation of photosynthesis, however it is only in acute conditions that chloroplast structures

are altered (Marschner, 2012). With prolonged deficiency, production of dry matter, photosynthetic activity and chlorophyll content are all significantly decreased.

A shortage of carbohydrates and reduction of lignin concentration due to Mn deficiencies, causes changes to root morphology and reduces root growth. The reduction in lignin content is thought to be a contributing factor leading to higher susceptibility to freezing temperatures and root-infecting pathogens such as take-all in wheat (Marschner, 2012).

1.3.6 Molybdenum

1.3.6.1 Role of nutrient in plant development

Of all the micronutrients, Mo is required in the lowest quantities within plant tissues. It is involved in a variety of redox reactions within plants when coupled with the pterin complex MoCo (Kaiser et al., 2005). The cofactor MoCo allows the correct anchoring and positioning of molybdenum within an enzyme to facilitate its interaction with other components of the electron-transport chain in which the enzyme participates (Barker & Pilbeam, 2007). Within enzymes, Mo moves between three oxidative states allowing two electron transfer reactions to occur (Marschner, 2012).

Only a small number of enzymes in higher plants has been found to contain Mo: nitrate reductase, xanthine dehydrogenase, aldehyde oxidase and sulphite reductase, although its role as a cofactor in nitrogenase in N_2-fixing bacteria is also important for legumes (Marschner, 2012).

1.3.6.2 Symptoms of deficiency

The characteristic phenotypes of Mo deficiency include mottled lesions on leaves and distortion of leaf morphology commonly referred to as ‘whiptail’, caused by reduced differentiation of vascular tissues early in leaf development (Barker & Pilbeam, 2007). Other symptoms often appear similar to N deficiency due to the role of Mo in N metabolism (Kaiser et al., 2005). The high mobility of Mo within the xylem and phloem means symptoms can be observed over the whole plant. Legumes often suffer more severely from Mo deficiency which

can lead to reductions in the weight and quantity of root nodules as well as stunted growth and chlorosis (Barker & Pilbeam, 2007).

1.3.7 Nickel

1.3.7.1 Role of nutrient in plant development

The recognition of Ni as an essential micronutrient has only been determined in the last 50-60 years. It is essential in the structure and catalytic function of urease (Marschner, 2012), an enzyme which metabolises urea into carbon dioxide and ammonia and may also have a protective role against pathogens (Hänsch & Mendel, 2009). Furthermore, in Ni deficient conditions barley plants were unable to produce viable seed suggesting that Ni has a role in grain filling and maturation processes (Brown et al., 1987).

1.3.7.2 Symptoms of deficiency

Nickel deficiency is uncommon, with toxicity presenting more of a problem with this element, however it has been seen to reduce shoot growth in barley, oats and wheat (Barker & Pilbeam, 2007). In addition, toxic accumulation of urea at leaf tips has been observed in Ni deficient conditions (Hänsch & Mendel, 2009).

1.3.8 Zinc

1.3.8.1 Role of nutrient in plant development

Zn is an integral component of enzyme structures and activities. Within enzymes, Zn has four functions; catalytic, structural, co-catalytic and acting at the protein-interface to help determine the activity of the enzyme (Marschner, 2012). Due to the Zn requirement of many enzymes, it is essential for a wide range of activities within plants including; cytochrome and nucleotide synthesis, chlorophyll production, maintenance of membrane activity and increased rate of seed maturation (Barman et al., 2018). Alcohol dehydrogenase is unusual in containing two Zn atoms, one with a structural function and the other a catalytic function allowing the reduction of acetaldehyde to ethanol. Other notable enzymes requiring Zn include RNA and DNA polymerase (Marschner, 2012).

Zinc finger proteins facilitate DNA binding of transcription factors and protein-protein interactions as well as controlling the proliferation and differentiation of cells (Sturikova et al.,

2018). They are also present in plant resistance proteins allowing effector-triggered immune responses to occur (Cabot et al., 2019).

1.3.8.2 Symptoms of deficiency

Zinc deficiency is common among plants grown on highly weathered acidic soils and calcareous soils (Barker & Pilbeam, 2007) with reports that globally approximately 30 % of cultivated soils are Zn deficient (Cakmak et al., 2017).

The most distinctive symptoms of Zn deficiency include stunted growth and a dramatic reduction in the size of developing leaves, commonly referred to as 'little leaf'. In acute conditions shoot apices die off. These symptoms are frequently combined with either highly contrasting or diffusive chlorosis. Most of the visible symptoms of Zn deficiency are indications of oxidative stress caused by increased generation of ROS and reduced activity of detoxification systems (Marschner, 2012). Reduction in protein production leads to an accumulation of amino acids within the plants (Barker & Pilbeam, 2007). Additionally sugars and starches also often accumulate in Zn deficient plants (Marschner, 2012).

1.4 Soil availability of plant nutrients

With the exception of N, all of the nutrients described above originate from rocks in the earth's crust. The soil gains these mineral nutrients through disintegration and decomposition of these parent materials (Vasey, 2002) through processes such as weathering and the activity of microorganisms (White, 2009). Despite the fact that most of the nutrients required by plants can be found within the soil, they are often in forms which are unavailable for plant uptake. Available forms of nutrients are soluble and therefore prone to leaching and erosion.

Soils are able to hold a reserve of nutrients due to the negative charges found on the surfaces of fine soil particles. These negative charges are able to attract and bind cations in a reversible reaction. The number of cations a soil can store on its surface is referred to as the cation exchange capacity (CEC) (White, 2009). This form of soil nutrient storage remains in equilibrium with the nutrients available in the soil solution and therefore is often referred to as the exchangeable fraction.

Nitrogen, P and K are usually the most limiting nutrients respectively (Vasey, 2002). Increasingly S is becoming more of a problem as reductions in environmental pollution have led to a decrease in depositions from these sources (Webb et al., 2016).

Nitrogen is the most common limiting factor for plant growth and development (Mcallister et al., 2012) as soils have poor retention of oxidized forms of N (Barker & Pilbeam, 2007). The global consumption of N fertilisers continues to increase in order to rectify these deficiencies. Whilst soils can retain some ammonium ions the concentrations are low ranging from 20-200 µM, although rice paddy soils often contain higher levels of ammonium to nitrate due to limited nitrification action in anaerobic soils (Marschner, 2012).

Phosphorus is taken up by plants from soil as inorganic orthophosphate (Pi), however this chemical form has low availability and mobility in most soils. Pi is rapidly converted in the soil to less available forms, due to its affinity with the cations Ca and Mg in calcareous soils and Al and Fe in acidic soils (Herrera-Estrella & López-Arredondo, 2016). It is estimated that approximately 50 – 70 % of global agricultural land is P deficient (Cakmak, 2002; Heuer et al., 2017). In combination with this, only 10-20 % of applied P fertilisers is absorbed by plants, with the rest being quickly transformed into unavailable forms. (Cakmak, 2002). Furthermore, P rock reserves from which inorganic P fertilisers are derived are a swiftly diminishing commodity, with generous estimates suggesting they can last 300-400 years (Heuer et al., 2017) and more conservative estimates, 50-150 years (Schröder et al., 2011).

Globally, soil K depletion is an increasing problem and restoration of K fertility would require a large increase in the world's current potash production (Manning, 2010). Moreover, inadequate application and imbalanced fertiliser regimes are leading to further declines in soil fertility in many parts of the world (Sheldrick & Lingard, 2004).

Recently S deficiencies have become more common, in part due to the reduction of atmospheric pollution with the introduction of scrubbing technologies in power plants. Between 1970-2010, UK SO_2 emissions decreased by 94 %, with further reductions anticipated as coal power stations are decommissioned (Webb et al., 2016). Furthermore, a reduction in the use of manure and an increased preference for N and P fertilisers such as urea, triple superphosphate and ammoniated phosphates which, unlike single superphosphate or

ammonium sulphate, do not contain any S (Ceccotti, 1996) have compounded the problem. Increasingly soils across the globe are facing S deficiencies with 42 % of Indian soils and 30 % of Chinese soils being recognised as S deficient (Singh Shivay et al., 2014).

Micronutrient concentrations and availability varies between soil types. Zinc deficiencies are particularly widespread globally, with estimates that 50 % of cereal are grown on soils with limiting Zn levels (Alloway, 2008). Furthermore it is reported that approximately 30 % of cultivated soils are Zn deficient (Cakmak et al., 2017). Fe is also often limiting to plant growth as it frequently forms insoluble complexes in aerobic soils with neutral to alkaline pHs. It is estimated that Fe limitation may affect up to 30 % of global soils (Morgan & Connolly, 2013).

1.5 The importance of microbial communities in the rhizosphere and their contribution to plant nutrition

The availability of plant accessible soil nutrients is greatly influenced by the soil microbial communities surrounding the plant root. Many plant-microbe interactions occur within the rhizosphere, a narrow region of soil surrounding the root (Philippot et al., 2013). Whilst total soil microbial diversity is thought to range up to 10 million species (Doornbos et al., 2012), rhizosphere microbial diversity contains up to 30 thousand species with approximately 10 billion microbial cells per gram of root (Berendsen et al., 2012). It is as yet undetermined whether edaphic or plant selection is the largest driving factor behind the composition of root-associated microbial communities, however the importance to the plant of these microbial communities, can be seen in the large quantities of resources they invest in recruiting microbes to this region of soil. Rhizodepositions in the form of root exudates as well as old root border cells, can contribute 5-30 % of the total carbon fixed by a plant (Jacoby et al., 2017). This investment not only provides a valuable nutrition source for the microbes occupying the rhizosphere, but is also beneficial for plant growth through microbial activities that solubilise soil nutrients (Haro & Benito, 2019), facilitates nutrient absorption from areas of the soil unreachable for the plant (Clark & Zeto, 2000), alters plant hormone signalling (Haro & Benito, 2019) and by competing with soil pathogens thereby reducing pathogen loads to plants (Sammauria et al., 2020).

Arbuscular mycorrhizae are a prime example of the phenomena described above. The production and release of phosphatases from the fungal hyphae provide access to organic P forms previously unavailable to the plant (Tarafdar & Marschner, 1994). Additionally, the small diameter of the fungal hyphae allows access to soil pores and cavities which otherwise would have been impenetrable to the plant. Furthermore, fungal hyphae have the ability to transfer nutrients over longer distances than roots allowing them to reach beyond depleted soil regions. Although most studies focus on mycorrhizal provision of N and P, there are a few which investigate the uptake of K, Ca and Mg. Most of these studies show inconsistent results with increases, decreases and no effect having been observed, however in general, plants in mycorrhizal symbioses see an improved acquisition of these nutrients, especially in acidic soils (Clark & Zeto, 2000).

Rhizobia are another noteworthy example of microorganisms interacting with plants to provide nutrition. The term rhizobia incorporates not only bacteria of the genus *Rhizobium* but also bacterial species capable of N fixation and nodulation in association with leguminous plants (Willems, 2006). Colonization of legume roots by these bacteria causes the deformation and curling of root hairs and eventually causes nodule formation (Hirsch et al., 2001). Within this plant-bacteria symbiosis, the rhizobia species provide N to the plant through their ability to fix N and in exchange receive carbon and other elements required for their metabolism from their associated legumes (Udvardi & Poole, 2013).

In addition to improved nutrient availability, root-associated microbial communities can impact plants through changes to yield quality and quantity, tolerance to biotic and abiotic stresses as well as alterations in timing of lifecycle stages (Lareen et al., 2016). Some arbuscular mycorrhizal fungal species have been show to help reduce salt stress in plants by assisting in increased K uptake whilst reducing Na translocation to shoot tissues (Porcel et al., 2016).

1.6 Using fertiliser to optimise plant nutrition

1.6.1 Historical fertiliser use

Over the millennia, farming has needed to restore soil fertility to ensure stable crop production. Many civilisations have used floodplains to grow their crops, providing irrigation

and in doing so have renewed soil fertility through the deposits laid down in this process. Crop rotations have also been and remain a widespread practice to allow soil regions to recover from the growth of crops (Leigh, 2004).

Fertilisers provided a more flexible way of reintegrating natural fertility than complex rotations. Artificial fertiliser usage began toward the end of the nineteenth century, particularly with the development of the Haber-Bosch process and their worldwide consumption rocketed after 1950 (Federico, 2005).

1.6.2 Organic fertilisers

Organic fertilisers include farmyard manures from cattle and pig slurry as well as poultry manure and sewage sludge. Slurries are mixtures of dung, urine and farmyard washings which can be combined with straw to produce farmyard manures. Typically, they are low in macronutrients when applied at recommended rates, although micronutrient composition can be relatively high. Whilst their nutritional benefits may be low, they also provide large quantities of organic matter which can improve soil structure and thus improve soil nutrient supply (White, 2009). In addition they have also been reported to increase soil microbial activity and diversity which can also affect plant growth (Walling & Vaneeckhaute, 2020).

Green manures and composts are forms of nutrient addition from plant materials. Green manures involve cultivating a quick-growing leaf crop – often a leguminous species to supply fixed N – which is ploughed back into the soil prior to crop planting. The green manure crop can reduce N leaching as these plants take up N and then slowly release it back through decomposition after ploughing (Cherr et al., 2006). Composts are produced by increasing the rate of humification of plant residues in moist, well-aerated heaps (White, 2009). Similar to farmyard manures, composts can have variable composition of nutrients.

1.6.3 Inorganic fertilisers

Mineral fertiliser use has increased rapidly since the green revolution, with a 10-fold increase in N fertiliser usage between 1961 – 2018, and P and K fertiliser use nearly quadrupling in that time (*FAOSTAT*). Commercial fertilisers can be applied to the soil as a spray or as granules or prills, the latter options being either broadcast on the soil surface or applied

with the seed or in bands in the soil. Foliar applications can also be used to avoid complex soil chemical interactions as well as reducing the energy costs needed for transport from root to shoot (Bindraban et al., 2015). Foliar sprays can also be used to target specific nutritional problems, for example boric acid and sodium borate can be used on perennial crops such as fruit trees, to improve reproductive success (Barker & Pilbeam, 2007).

Prior to 1909, the most common form of N fertilisation was through manures and ammonia derived from coal gas, however the development of the Haber process allowed the production of N in the form of ammonia. The most commonly used dry N fertiliser is urea, however ammonium nitrate and anhydrous ammonia are also popular options.

Both P and K fertilisers are usually derived from rock reserves. Globally the ores containing K are limited in distribution with the majority of the world's K fertilisers sourced from mines in the Northern Hemisphere, particularly Canada, Russia and Belarus (fig. 1.2) (Manning, 2010). Across the world, potassium chloride (KCl), also referred to as muriate of potash (MOP) is the favoured K fertiliser as it is cheap, with prices in 2019 averaging £252/tonne (AHDB, 2021) and has a high K content. In some areas, potassium sulphate (K_2SO_4) also referred to as sulphate of potash (SOP) is used, especially when there are soil S deficiencies in the area or when crops are sensitive to chloride (Manning, 2010), however it is more expensive than KCl with prices between 2015-2017 averaging £360/tonne (converted from US$ to £ from Zhou et al., 2019).

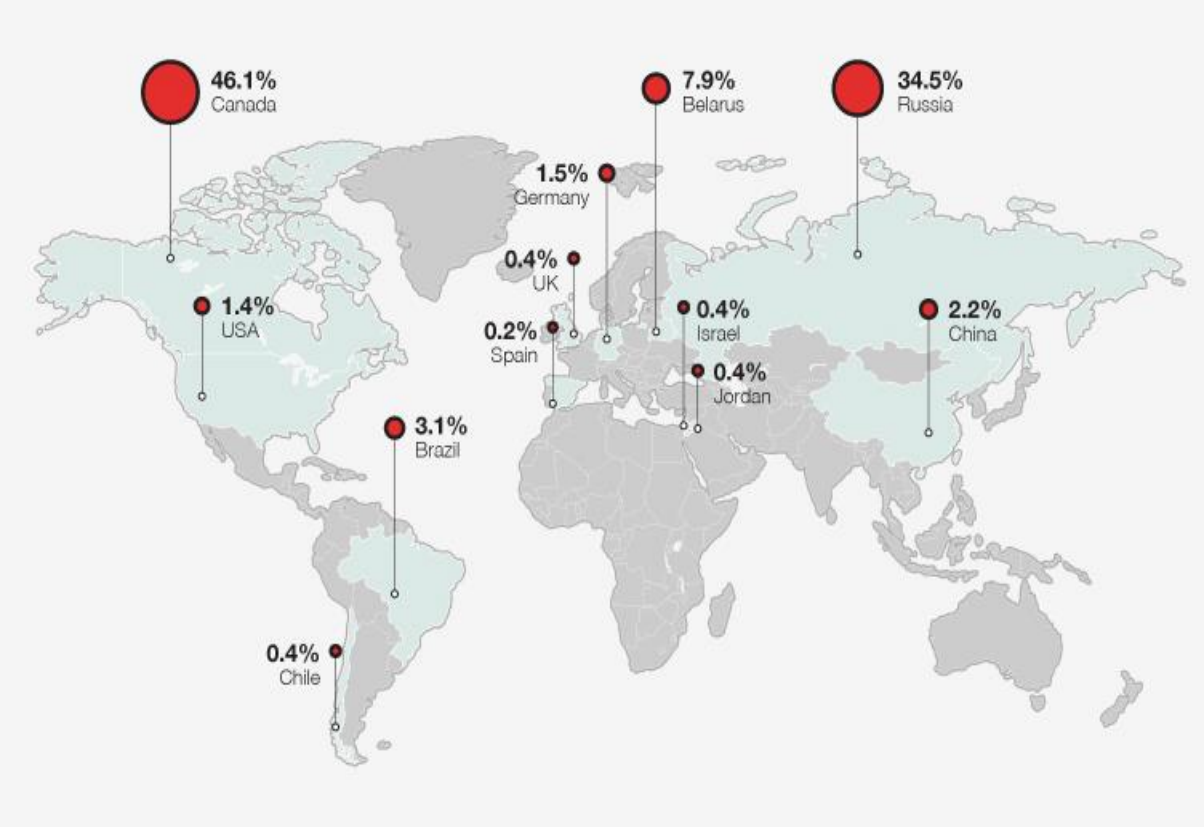


Figure 1.2 Map of global distribution of potash reserves (Uralkali, 2017)

Water soluble P fertilisers are produced by reacting phosphate rocks with sulphuric or phosphoric acids, whereas ammonium phosphates pass anhydrous ammonia through phosphoric acid for their production (Barker & Pilbeam, 2007). Ammonium phosphates are the most popular choice, occupying 48.7 % of global consumption and provide both N and P (Walling & Vaneeckhaute, 2020). Single and triple super phosphates (SSP and TSP respectively) are also widely used worldwide (Barker & Pilbeam, 2007).

Traditionally, sulphate containing fertilisers such as ammonium sulphate, single super phosphate, gypsum, potassium sulphate and magnesium sulphate were used as soil applications (Fuentes-Lara et al., 2019). Increasingly however, the use of elemental S, bentonite S and micronized S are becoming more popular and are examples of slow release fertilisers. For example, bentonite S usually contains 90 % S and 10 % bentonite. Upon contact with the soil the bentonite begins absorbing water from the soil causing the clay to swell and thus fragment the fertiliser granule, producing smaller elemental S particles which can subsequently by oxidised by microbial species (Prasad et al., 2018).

The most common Ca fertiliser applications are chalk or lime; however, these tend to be applied to improve soil drainage and increase soil pH. Single superphosphate and TSP are also incidental ways in which Ca is applied to the soil as they are primarily applied for their P content. Similarly, calcium sulphate (gypsum) is often used as a fertiliser but usually for its S content (Barker & Pilbeam, 2007).

Common Mg fertilisers can be divided into two categories; soluble sources and semi-soluble sources. Soluble sources include minerals in which the Mg is in the form of $MgSO_4$. Slow release Mg fertilisers include dolomite, magnesite and calcined magnesite which can help to reduce leaching, however often do not deliver practical quantities of plant available Mg to crops (Senbayram et al., 2015).

Micronutrient fertilisers can be applied individually or in mixed fertilisers. They tend to be in the form of chelates or as sparingly soluble salts or oxides. As well as soil applications, foliar sprays are often used for micronutrient efficiencies as they allow immediate uptake by the plant region affected by the deficiency (White, 2009).

1.6.4 Benefits of fertiliser use

Balanced fertilisation is integral to maintaining high yields and various guidelines have been developed including the 4R Nutrient Stewardship guide to help farmers achieve best practices on their land. These guidelines help to identify not only the correct rate of application, but also the right source of nutrients, timing of application as well as ensuring that efforts are made to keep the nutrient in a position that is most useful for the crop (Johnston & Bruulsema, 2014).

Interactions between nutrients can either be synergistic, leading to a greater growth response than the sum of the nutrient's individual effects or antagonistic where a combination of nutrients leads to negative effects on yield. Synergistic responses can be encouraged by mixing nutrients and applying them at the same time. Most macronutrients have synergistic interactions which have the capability to lead to 3-fold increases in yield compared to the predicted yield. Optimising fertiliser use efficiency requires maximising nutrient synergies and reducing antagonistic nutrient interactions (Rietra et al., 2017).

Different crop species require varying quantities of each nutrient (table 1.1). For example, members of the Brassicaceae family e.g. oilseed rape, require higher quantities of S than many cereal crops such as rice and wheat. Therefore, fertilisation requirements must be amended for different crop species. Therefore, soil sampling and careful records of field usage can be integral for optimal fertiliser applications.

As well as crop species requiring different nutrient profiles, different crop genotypes can also often have distinct nutrient requirements. The capability of different genotypes to take up and utilise nutrients is referred to as their nutrient use efficiency (NUE). The use of crop genotypes which have a high NUE can lead to improved yields with the same or lower applications of fertilisers.

Table 1.1 – Sufficient concentrations of nutrients in plant tissues for a variety of crop species

	Sufficient Concentration of Nutrient In Plant (% of dry weight)			
Crop	**K**	**S**	**Mg**	**Ca**
Rice	2 - 3*	0.2 – 0.3	0.15 – 0.3	0.2 – 0.6*
Wheat	3.5 – 5.5	0.15 – 0.4	0.15 – 1	0.47
Barley	3.5 – 5.5	0.15 – 0.4	0.15 – 0.4	0.73
Maize	2 – 3.5	0.21 – 0.5	0.13 – 1	0.92
Oilseed rape	2.8 – 5	0.35 – 0.47	-	-
Soybean	2.5 – 3.7	0.21 – 0.4	0.25 – 1	-
Potato	5 – 6.6	0.19 – 0.36	0.5 – 2.5	0.45
Sugarcane	-	0.14 – 0.2	0.1 – 0.2	-
Alfalfa	2.5 – 3.8	0.26 – 0.5	0.3 – 1	1.5
Cassava	1.7 – 3.5	0.3 – 0.4	0.25 – 0.6	-
Cotton	2.5 - 3	0.2 – 0.25	0.3 – 0.9	-

Data from Barker and Pilbeam (2007), except where starred when data if from Dobermann and Fairhurst (2000)

1.6.5 Problems with fertiliser use

Nutrient imbalances can be seen worldwide with only 10 % of croplands accounting for 32 % of the world's surplus nitrogen addition and 40 % of surplus phosphorus addition. Areas of nutrient use deficiency can also be identified (Foley et al., 2011), providing targeted regions

for improvement in efficient nutrient applications. These inefficiencies in nutrient application, can lead not only to unnecessary expense on the part of overapplication of fertilisers, but also less healthy plants, leading to potential yield losses. Overapplication of fertilisers can have serious environmental impacts with both excess N and P leading to eutrophication problems and degradation of wildlife habitats as well as the release of the greenhouse gas, nitrous oxide (DeFries et al., 2015).

Underapplication or imbalanced application of fertilisers can lead to lower yields and inefficient use of fertilisers. Imbalanced applications can lead to antagonistic nutrient interactions where a combination of nutrients leads to negative effects on yield. For example, in K deficient soils, increased N fertiliser applications will not lead to higher yields as optimal osmotic capacities cannot be maintained due to insufficient K, therefore leading to reduced growth capabilities (Milford & Johnston, 2007). The production of new cells and increased shoot growth caused by nitrogen leads to an increased requirement for K. The growth of new cells, each of which contain a vacuole, requires higher water content and thus an increase in K for maintenance of osmotic potentials within the cell to retain sufficient cell turgor.

Similarities in chemical properties can also lead to nutrient interactions, as plant transporters are often unable to differentiate between similar ions. For example, P3A-type H-ATPases can transport Na, K, Ca and Zn, therefore if one ion has an elevated soil concentration it may outcompete other nutrients for uptake. Antagonistic nutrient responses can be avoided by using different forms of fertiliser application for the different nutrients, for example applying some fertilisers to the soil and some as foliar sprays (Rietra et al., 2017).

1.6.6 Improvements in fertiliser technology

The disruption of natural processes due to the low assimilation of fertiliser applications leads to the deterioration of aquatic and terrestrial ecosystems. To reduce this disruption, a range of slow releases and controlled release fertilisers (SRFs and CRFs respectively) has been developed. Release of nutrients from these fertilisers is controlled through chemical and physical characteristics and can allow the rate of nutrient release to be more closely synchronised with a plant's requirements throughout its growth (Ali & Danafar, 2015). SRFs include synthetic and natural materials for which rate of nutrient release is retarded, often due

to low solubility in water or because release is dependent on microbial activity (White, 2009). Whilst nutrient release is slowed in SRFs, the rate, pattern and duration of release is not well controlled and can be dependent on environmental conditions (Sempeho et al., 2014). CRFs are synthetic fertilisers which can feature a core of fertiliser encapsulated by a hydrophilic layer or the nutrients being incorporated into a matrix, and in some cases a combination of these approaches. In coated CRFs, the outside layer can be made up of sulphur, phosphates, silicates, polyethylene, poly vinyl chloride, poly lactic acid or wax (Ali & Danafar, 2015). These coatings help control water penetration to the core nutrient fertiliser and therefore regulate their rate of dissolution (Sempeho et al., 2014). The most common CRFs are sulphur and polymer coated urea (Fu et al., 2018).

The benefits of SRFs and CRFs can be seen in reduction of mineral nutrient losses through synchronization of nutrient release to match plant demands as well as in reduced labour and energy costs, as fewer applications of fertiliser are required. Additionally, storage and handling can be more convenient in the case of coated fertilisers as their coatings can reduce the hygroscopic nature of the chemicals making them less sensitive to humidity and caking problems (Timilsena et al., 2015). It has also been suggested that coating ammonium nitrate can reduce the likelihood of explosions as it leads to higher thermal stability (Ali & Danafar, 2015).

Despite the advantages of these fertiliser technologies, uptake is very low – less than 1% of total fertiliser consumption. The major barrier to uptake of these fertilisers is cost, with estimations of polymer coated fertilisers being 8-12 times higher than standard NPK fertilisers. Lack of growers' experience and familiarity with these fertilisers can also make it difficult to choose the correct CRF or SRF for their situation (Timilsena et al., 2015).

1.6.7 Polyhalite as an alternative K fertiliser

Polyhalite is a hydrated evaporate mineral (Smith et al., 2014) containing potassium, magnesium, calcium and sulphate with the following composition $K_2SO_4 \cdot MgSO_4 \cdot 2CaSO_4 \cdot 2H_2O$ (Fraps & Schmidt, 1932). In addition to the four macronutrients, K, Mg, Ca and S that polyhalite provides, it also contains the beneficial element Na and trace quantities of the micronutrients; B, Zn, Mn, Mo, Fe and Cu. Globally, polyhalite is not an unusual mineral; however normally it is

found as a small component of sequential marine evaporations (Smith et al., 2014). Deposits of polyhalite were discovered in Yorkshire in 1939 but were overlooked due to the sylvite beds, the mineral form of KCl, found around the same time (Kemp et al., 2016). It has recently been established that there is a layer of more than 2.5 billion metric tonnes available, rendering this polyhalite deposit to be the largest known in the world. The area of the proposed mining project can be seen in figure 1.3. Additionally, these deposits are a very high grade (in some places >99% polyhalite) meaning the only processing required before retailing is crushing and sizing (Kemp et al., 2016). Further deposits of polyhalite have subsequently been discovered in China (Shang et al., 2021). Whilst polyhalite can be processed further to produce potassium sulphate, magnesium compounds and gypsum, it can also be used directly as a long-release fertiliser (Barbarick, 1989; Smith et al., 2014). The slow-release nature of polyhalite has the potential to improve K use efficiency (KUE) as it prevents leaching and provides prolonged nutrient release. Additionally, polyhalite may be a preferable K fertiliser compared to KCl for chloride sensitive plants.

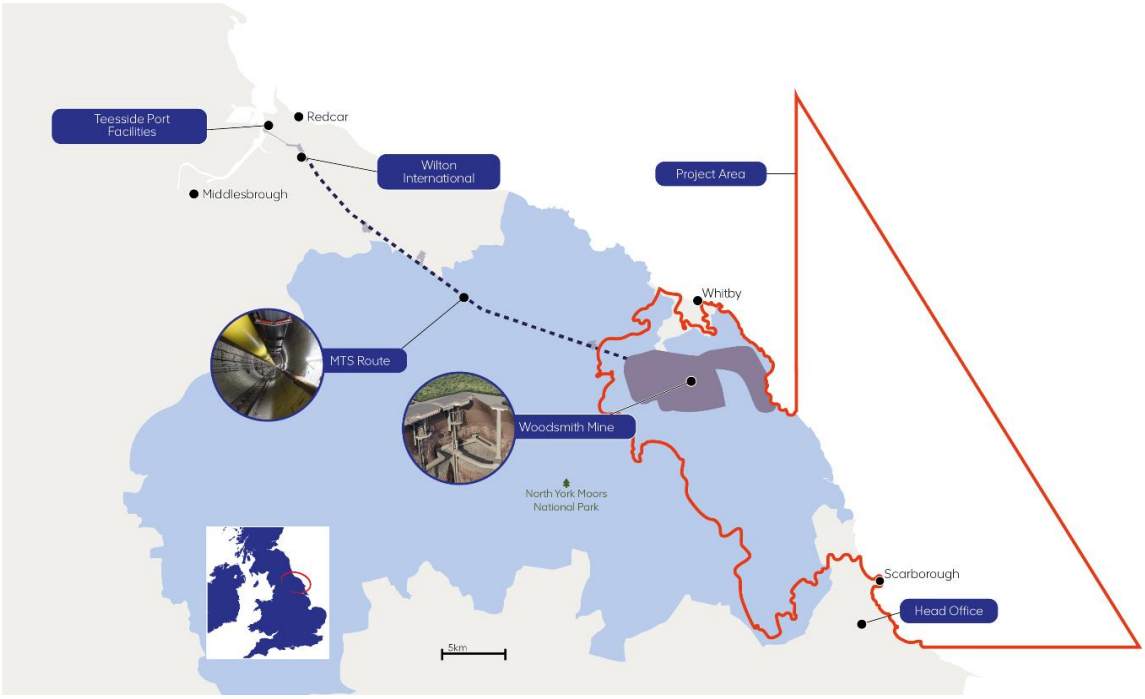


Figure 1.3 - Map of North Yorkshire with the proposed mining site (area within the red line) of polyhalite by AngloAmerican in and off the coast of North Yorkshire (AngloAmerican, 2020)

1.7 The global importance of rice and its use as a model organism

1.7.1 The global importance of rice as a crop

Globally there are two species of cultivated rice, *Oryza sativa* and *O. glaberrima. Oryza glaberrima* originated and is grown in Africa whereas *O. sativa* originated in Asia and is the staple food for half the world's population, with global paddy rice covering over 162 million hectares and producing 755 million tonnes in 2019 (FAOSTAT). Many families in developing countries rely on rice for both employment and income with over 200 million households having their main source of income and often main source of food from the crop (Muthayya et al., 2014). Despite the importance of rice as a crop, the actual yield in many countries does not come close to the yield potential, with estimates of Indian rice crop yields only reaching 53 % of the yield potential (Lobell et al., 2009).

There are an estimated 140 000 different landraces and elite cultivars of *O. sativa* providing a large amount of genetic variation (Bellon et al., 1998). Different subspecies of rice include: japonica and indica, lowland and upland as well as glutinous and non-glutinous. The most significant genetic differentiations can be seen between the indica and japonica ecotypes and there is considerable reproductive isolation between them. In addition, substantial differences can be seen in phenotype, grain qualities and characteristics between the ecotypes. Indica cultivars have a higher heat tolerance and therefore are mainly found at low altitudes in tropical and subtropical regions, whereas japonica cultivars tend to be grown in temperate climates, although they can also be cultivated in mountainous regions of lower latitudes (Lu et al., 2009).

1.7.2 Rice as a model organism

Whilst *Arabidopsis thaliana* is widely recognised as an important plant model species, rice is also an excellent model organism due to its substantial pre-existing genetic, molecular and genomic resources. In addition, its position within the Poaceae family provides synteny with many other cereal crops (Rensink & Buell, 2004) as well as being a major crop itself. Furthermore, it has a relatively small genome compared to other cereal species (Goff et al., 1999).

1.7.3 General fertilisation of rice

Rice crops take up approximately 20 kg N, 4.8 kg P, 25 kg K, 3 kg S, 7 kg Ca and 3 kg Mg per tonne of rough rice (i.e. whole rice grains pre-processing) produced (Roy et al., 2006). Rice yields are often constrained through poor nutrient management due to unbalanced fertiliser regimes which favour N above all other nutrients, as the yield effects can be seen directly by the farmer (Dobermann et al., 1996). In many cases, N is the main or only nutrient applied to rice crops, as soil N is insufficient in most areas. Often this is exacerbated by governmental recommendations for fertiliser application which will usually include N and P but may either not include K or only suggest a very low rate of application (Bijay-Singh & Singh, 2017). The average N fertiliser rate for paddy rice is 128.1 kg N ha^{-1} (Zhong et al., 2016) although it varies greatly between countries and regions. Potassium applications tend be between 15-20 kg K ha^{-1}, which are insufficient for continued crop growth, leading to increasing areas of K-deficient soil (Dobermann & Fairhurst, 2000). Soil P varies from being deficient to overabundant and fertiliser tends to be applied at 15-20 kg P ha^{-1} (Witt et al., 1999).

1.7.4 Potassium fertilisation of rice

Potassium chloride, is the favoured K fertiliser for rice due to its low cost and high K content. Where soil S deficiencies are prevalent, K_2SO_4 may be preferred. Potassium is frequently only applied prior to or at sowing, but split applications do also occur and can help to increase the number of spikelets and panicles (de Datta, 1981). If split applications of K are made, they are usually at or before planting, panicle initiation (PI) and first flowering. (Dobermann & Fairhurst, 2000).

1.8 Objectives of study

The benefits of polyhalite as a K fertiliser have been claimed by a number of studies, however these studies are few in number and often limited to field trials in which fertiliser treatments are not fully balanced nutritionally (Yermiyahu et al., 2017; da Costa Mello et al., 2018b; Barbier et al., 2017; Keren-Keiserman et al., 2019; da Costa Mello et al., 2020; Zhou et al., 2019; Pavuluri et al., 2017; Dal Molin et al., 2020). Whilst such studies provide valuable information about how farmers compare fertilisation regimes in the field there is an urgent need to determine how K sources compare when the nutrient composition of treatments are fully balanced and controlled. For the context of this thesis, balanced fertiliser treatments are

those which apply the same quantities of each nutrient, whereas an unbalanced fertiliser treatments directly compare K fertilisers with no additional nutrients added to ensure each nutrient is applied equally. For example, in a balanced experiment comparing K_2SO_4 and polyhalite, the K_2SO_4 would have additional salts containing Mg and Ca applied to match it to the nutrients applied by polyhalite, however in an unbalanced experiment, these additional salts would not be added, instead the two fertilisers would have the same rate of K applied and all other nutrients would be not be matched.

The main focus of this study is therefore the comparison of polyhalite with other K fertilisers and the central objectives of this study are to:

1. Evaluate whether polyhalite affects rice growth traits and nutrient content compared to KCl and K_2SO_4, using both balanced and unbalanced fertilisation regimes
2. Assess how different K fertilisers affect growth and nutrient composition of rice in a genotype-specific manner.
3. To evaluate whether K fertiliser types affect the composition of soil microbial communities.

The first objective involved growing plants in hydroponics as well as in sand which allowed easy manipulation of the growth medium, thus improving reproducibility. Plants were also grown in soil to provide a more physiologically relevant growth medium and to allow comparison of balanced and unbalanced fertiliser regimes.

The second objective used a range of rice cultivars that was previously shown to vary in potassium use efficiency (KUE). If there are differences in uptake between cultivars, this objective may identify ways in which to improve rice fertilisation by tailoring K fertiliser types to specific cultivars or as potential avenues for breeding improved KUE in crops.

Finally, there is an increasing recognition of the importance of soil microbial communities on crop growth and health, however few studies have investigated the interaction of inorganic fertiliser application on soil microorganisms and those that have, tend to focus on N and P fertilisers. The third objective therefore aimed to determine whether different K fertiliser regimes influenced the species diversity of these populations.

Chapter 2: Investigating the use of polyhalite as a K fertiliser in multiple growing media

2.1 Introduction

Slow-release and controlled release fertilisers are increasingly being investigated as approaches for improved plant nutrition due to their prolonged and gradual release of nutrients which often better mimic the release rates of organic materials (Kiran et al., 2010). A further advantage of the longer release profile of these fertilisers is the reduction of nutrients being leached from the soil, therefore bringing benefits both to the crop and to the surrounding environment by reducing the use of excess N and P fertilisers. Whilst K fertilisers do not cause environmental concerns, a reduction in leaching and the extended delivery time of the nutrient to a crop would increase K use efficiency (KUE) and therefore be beneficial.

The most common K fertilisers used on crops tend to be KCl and K_2SO_4 with the former being the preferred option mainly due to its low cost and high K content whilst the latter is favoured for use on chloride sensitive plants. Polyhalite may be a suitable alternative K fertiliser for farmers to utilise. It has a number of favourable features including an absence of Cl and slow-release chemistry. As well as providing the essential macronutrients K, S, Ca and Mg, polyhalite also contains the beneficial element Na and trace quantities of the micronutrients; B, Zn, Mn, Mo, Fe and Cu. Polyhalite can be used in a variety of forms but is primarily commercialised in granules approximately 2-4 mm in diameter which contain polyhalite powder bound with corn starch. Independent of fertiliser type, the method of K application can also influence the rate in which K moves through the soil and its levels of leaching. For example, suggestions have been made that topdressing with a granular or coated K fertiliser on sandy soils could be a beneficial way to reduce K leaching from the topsoil (Munson & Nelson, 1963).

Whilst slow-release and controlled-release fertilisers have been found to increase nutrient use efficiency (Morgan et al., 2009), improvements in crop yields are not consistently observed compared to conventional practices. When investigating the effect of 5 different slow-release N fertilisers on rice yields across two growing seasons (including sulphur-coated urea, 2 different polymer coated ureas, a humic coated urea and a diurea compound requiring

microbial and chemical processes for N release), Kiran et al., (2010) observed that 4 of the 5 fertilisers outperformed in yield compared to a urea control. In contrast, Wei et al., (2018) found that the three slow or controlled-release N fertilisers (polymer-coated urea, sulphur-coated urea and urea formaldehyde) they studied did not perform as well as the urea control treatment in terms of rice yield when fertilisers were applied before seedlings were transplanted. When the application was split, with one application before seedlings were transplanted and a second at tillering, only the urea formaldehyde slow release fertiliser provided a higher yield than conventional urea.

Increased cotton yields and improvements to crop quality have been seen through the use of a polymer coated KCl fertiliser blended with regular KCl, compared to regular KCl alone (Chen et al., 2021). Improved KUE and plant production in cotton were seen by Tian et al., (2017) when using a polymer coated KCl compared to K_2SO_4 and non-coated KCl. Maize yields have also been seen to increase when using a controlled release form of KCl blended with ordinary KCl (Li et al., 2020).

A number of studies have been performed comparing polyhalite to currently retailed single-salt K fertilisers (table 2.1). Whilst a number of these studies reported yield increases when polyhalite is used, they were not based on fully balanced fertiliser regimes to match the polyhalite profile, often with only the N, P and K being applied at the same rates. As polyhalite is a multi-nutrient fertiliser adding not only K but also S, Mg and Ca the absence of these nutrients from the fertiliser regimes makes it impossible to determine whether the additional nutrients from the polyhalite or another inherent property of polyhalite led to the yield increases. For example, Pavuluri et al (2017) reported an increased yield in maize plants grown in Tanzanian field conditions when using polyhalite compared to KCl or K_2SO_4 based fertilisers. However, no yield difference was seen when comparing polyhalite to a treatment containing KCl and kieserite, a compound containing K, S and Mg. In contrast, two other studies investigating maize reported no significant differences between plants grown with different K sources. Molin et al (2019) reported no significant yield differences in both of their experiments; one an unbalanced nutrients study comparing polyhalite with KCl and the other a balanced nutrients study comparing polyhalite with K_2SO_4 + kieserite + gypsum. Lillywhite et al (2020) also reported no significant differences in yield between plants fertilised with KCl, K_2SO_4

Table 2.1 – Treatments and crops used in polyhalite literature

Crop	Treatment				Reference
	Control	**KCl**	**K_2SO_4**	**Poly**	
Maize	1) No fertiliser 2) N + P	1) KCl 2) KCl + kieserite	-	x	Pavuluri et al 2017
Maize	-	x	K_2SO_4 + kieserite + gypsum	x	Molin et al 2019
Maize	N	1) KCl 2) KCl + gypsum	K_2SO_4 +gypsum	x	Lilleywhite et al 2020
Barley	N + P	x	x	x	
Tomato	N + P	KCl + SSP	1) K_2SO_4 2) K_2SO_4 + $MgSO_4$	x	Mello et al 2018a
Tomato	N + P	1) KCl 2) KCl + SSP	1) K_2SO_4 2) K_2SO_4 + $MgSO_4$	x	Mello et al 2020
Potato	N + P	x	K_2SO_4 + kieserite + gypsum	Poly + KCl	Mello et al 2018b
Tea	N + P	-	x	x	Zhou et al 2019

N + P: nitrogen and phosphorus

SSP: Single super phosphate (adds P and Ca)

Poly: polyhalite

Crosses show where the treatment was used with no additions, dashes are present when the treatment was not used.

or polyhalite. This study also investigated barley and found that that KCl treated plants had a lower yield than those in polyhalite or K_2SO_4 treated plants (Lillywhite et al., 2020).

Conflicting results have also been reported in tomato where marketable yield was significantly higher in polyhalite treated plants compared to all other treatments (da Costa Mello et al., 2018b). However another study using the same treatments in tomato found that whilst marketable yield was higher in polyhalite treated plants compared to the control treatment containing N and P only, no significant differences were seen between any of the K containing treatments (da Costa Mello et al., 2020).

A three year trial in tea comparing a no K fertiliser control, polyhalite and K_2SO_4 observed no significant differences in tea yields between treatments in the first year's harvest, however in the second year plants grown with K containing fertiliser treatments had higher yields than the no K control and in the final yield the polyhalite treated plants outperformed both of the other treatments (Zhou et al., 2019).

Very few studies have compared polyhalite with a fertiliser treatment that fully complemented the nutrition found in polyhalite. Those that have did not find yield increases between the fully balanced treatments in both tomato (Dal Molin et al., 2020) or potato (da Costa Mello et al., 2018a).

Despite the importance of nutritional content for plant health and the potential impacts on crop quality, not all of the aforementioned studies have investigated the nutritional content of the plants in their experiments. Those that have, have only focused on the macronutrients K, S, Mg and Ca with no consideration of the impact that changes in these nutrients may have on the wider nutritional status of the plant. In tea plants no significant differences were reported in K or S content between polyhalite and K_2SO_4 treatments, however both Mg and Ca contents were significantly increased in polyhalite treated plants compared to K_2SO_4 (Zhou et al., 2019).

In barley plants, polyhalite applications provided similar or increased grain K and S content to KCl and K_2SO_4 (Lillywhite et al., 2020). Similar results have been reported in maize where no differences were found in K content between polyhalite and other K containing treatments (Dal Molin et al., 2020; Lillywhite et al., 2020). Sulphur content in maize has also been reported with no significant differences between polyhalite and other K containing treatments (Dal Molin et al., 2020) however another study found one experiment produced no significant differences in S content within the plants whereas their other experiment found higher S content in both polyhalite and K_2SO_4 treated plants compared to KCl treated plants (Lillywhite et al., 2020).

In tomato plants K content has been inconsistent between treatments with one study finding higher foliar K in polyhalite treated plants than KCl treated plants (da Costa Mello et al., 2020). Another study found no significant differences in foliar K content at one field site, however at another location foliar and fruit K content was higher in polyhalite treated plants than KCl or K_2SO_4 treated plants (da Costa Mello et al., 2018b).

Previous experiments have predominantly been performed in the field (Lillywhite et al,. 2020; Pavuluri et al., 2017; Zhou et al., 2019; da Costa Mello et al., 2018a, 2018b, 2020), often at multiple locations or in multiple crop species, which causes additional confounding

variables due to differences in climate, soil type, altitude and pathogens. Controlled environment studies can reduce these external factors and in combination with fully balanced fertiliser regimes can help to provide more information about polyhalite's effectiveness as a K fertiliser.

In some cases, certain K fertilisers may be used due to availability or price and may simply be swapped in or out of fertiliser regimes due to these factors, without taking into consideration the other nutrients that each K fertiliser may provide. The observation of plants provided with different K sources without balancing the additional nutrients can help to provide more information about polyhalite's effectiveness as a K fertiliser in such circumstances.

As the studies performed to date have mostly focussed on fertiliser regimes which interchanged K sources without considering the additional nutrients that may be applied with each K fertiliser, it was important to determine how plants responded to the K sources when provided as a part of a fertiliser regimes with all nutrients equivalent between treatments i.e. a balanced fertiliser regime. To provide comparison to previous studies it was also necessary to include an experiment with different K sources without balancing the additional nutrients i.e. an unbalanced fertiliser regime, to provide more information about polyhalite's effectiveness as a K fertiliser in such circumstances. As well as comparing different K fertilisers it was also important to consider the different forms of polyhalite (i.e. powder or granules) and whether any of these forms caused improved growth or nutritional status. Furthermore, it was important to consider whether the corn starch binding agent within the polyhalite granules impacted plant growth of nutrient content.

In this study, polyhalite was tested in the glasshouse in both hydroponics and pots to determine its efficacy as a K fertiliser for rice plants. Pot experiments included balanced and non-balanced fertiliser regimes; the former to determine whether the chemistry of polyhalite altered plant nutrient uptake and the latter to determine how well polyhalite performed compared to the K fertilisers currently on the market. A number of different polyhalite forms were also compared to establish whether the binding agent had any effect on rice plants and to allow for comparison of the slow release nature of the granules compared to the more

immediate release of nutrients from the polyhalite in powder form. Rice growth, yield and nutrient content at different life stages were recorded to assess whether different K fertiliser regimes altered plant growth responses.

2.2 Materials and Methods

2.2.1 Growth Methods

2.2.1.1 Investigating the use of polyhalite as a potassium fertiliser in hydroponically grown plants

Rice (*Oryza sativa cv. Zhenshan 2)* seeds were sown and germinated in sand (Aggregate industries) with deionised water. After two weeks, 88 seedlings were transferred to 350 x 220 x 140 mm black plastic boxes filled with 9 litres of either a polyhalite based nutrient solution (Poly) or an adjusted yoshida based nutrient solution (AY1) (table 2.2) (Yoshida et al., 1976) each box containing 44 plants. The nutrient solution was changed weekly. Four plants from each treatment were weighed every week for the duration of the experiment. Ten plants were removed from each treatment at the following stages: “seedling” (35 d), “vegetative” (56 d), “flowering” (77 d), “grain” (98d) for destructive harvesting. This block of 88 plants was replicated 3 times at different times of the year to reduce any seasonal effects on results.

Table 2.2 – Nutrient concentrations for solutions used in experiments 2.2.1.1

Nutrient	Chemical providing nutrient	Concentration of nutrient in polyhalite (Poly) medium (mM/l)	Concentration of nutrient in Adjusted Yoshida medium (AY1) (mM/l)
N	NH_4NO_3	2.9	2.9
P	$NaH_2PO_4.2H_2O$	0.3	0.3
	H_3PO_4	-	-
K	Polyhalite (K_2O)	1	-
	K_2SO_4	-	1
	KCl	-	-
Ca	Polyhalite (CaO, $CaSO_4$)	1.1	-
		-	1
	$CaSO_4$	-	0.1
	$CaCl_2.2H_2O$		
Mg	Polyhalite (MgO, $MgCO_3$)	0.6	-
		-	0.6
	$MgSO_4.7H_2O$		
S	Polyhalite (SO_3, $CaSO_4$)	2.1	-
		-	0.5
	K_sSO_4	-	1
	$CaSO_4$	-	0.6
	$MgSO_4.7H_2O$		
Na	Polyhalite (NaCl)	0.2	-
	$NaH_2PO_4.2H_2O$	0.3	0.3
	Na_2SiO_3	0.25	0.25
	NaCl	-	0.2
Mn	$MnCl_2.4H_2O$	0.01	0.01
Mo	$(NH_4)_6.Mo_7O_{24}.4H_2O$	0.001	0.001
B	H_3BO_3	0.2	0.2
Zn	$ZnSO_4.7H_2O$	0.0002	0.0002
Cu	$CuSO_4.5H_2O$	0.04	0.04
Fe	$FeCl_2.6H_2O$	0.43	0.43

2.2.1.2 Investigating the use of polyhalite as a potassium fertiliser on plants grown in sand with a balanced fertiliser regime

Rice (*Oryza sativa cv. Zhenshan 2)* seeds were sown and germinated in sand with deionised water. After three weeks, 3 seedlings per treatment were transferred to 9 x 9 x 9 cm pots filled with 1.4 kg sand, with 3 pots per treatment. Pots were placed in circular 14 cm diameter, 4 cm high plastic boxes with a hole cut in the lid to surround the plant pot and were covered in black tape to reduce evaporation. Plants were fed after transfer and then every 2 weeks for 8 weeks after transfer to pots and were arranged in a complete randomised block. Fertiliser treatments (table 2.3) included 1) a control of 200 ml of an adjusted yoshida nutrient solution (AY2), 2) a polyhalite solution made from polyhalite powder (PP), 3) a polyhalite solution made with polyhalite granules (PGS) and 4) polyhalite granules (PG) added to the surface of the sand. All polyhalite based treatments were matched to AY2 using N, P and micronutrients (NPM), either adding them to the solutions of treatments 2 and 3 or adding them additionally to the pot for treatment 4, with 200ml of solution added at each fertilisation timepoint. Three complete sets of three plants for the four treatments were grown to allow for destructive harvesting at three times points; 14 days after final fertiliser addition, after the first panicle fully emerged and finally, one month after the first panicle had fully emerged. This block of 36 plants was replicated 3 times at different times of the year to reduce any seasonal effects on results.

Table 2.3 – Nutrient concentrations for solutions used in experiments 2.2.1.2 and 4.2.1.1

Nutrient	Chemical providing nutrient	Concentration of nutrient in Poly Powder medium (mM/l) for pots (PP)	Concentration of nutrient in Adjusted Yoshida medium (mM/l) for pots (AY2)	Concentration of nutrient in Poly Granules medium (mM/l) for pots (PGS)	Concentration of nutrient in N, P and micronutrients medium (mM/L) for pots (PG)
N	NH_4NO_3	14.5	14.5	14.5	14.5
P	$NaH_2PO_4.2H_2O$	1.5	1.5	1.5	1.5
K	Polyhalite (K_2O) K_2SO_4	5 -	- 5	5 -	(provided as granules 0.3g/feed)
Ca	Polyhalite (CaO, $CaSO_4$) $CaSO_4$ $CaCl_2.2H_2O$	5.5	- 5 0.5	5.5 - -	- - -
Mg	Polyhalite (MgO, $MgCO_3$) $MgSO_4.7H_2O$	3 -	- 3	3 -	- -
S	Polyhalite (SO_3, $CaSO_4$) K_sSO_4 $CaSO_4$ $MgSO_4.7H_2O$	10.5 - - -	- 2.5 5 3	10.5 - - -	- - - -
Na	Polyhalite (NaCl) $NaH_2PO_4.2H_2O$ Na_2SiO_3 NaCl	1 1.5 1.25 -	- 1.5 1.25 1	1 1.5 1.25 -	- - - -
Mn **Mo** **B** **Zn** **Cu** **Fe**	$MnCl_2.4H_2O$ $(NH_4)_6.Mo_7O_{24}.4H_2O$ H_3BO_3 $ZnSO_4.7H_2O$ $CuSO_4.5H_2O$ $FeCl_2.6H_2O$	0.05 0.005 1 0.001 0.001 0.2	0.05 0.005 1 0.001 0.001 0.2	0.05 0.005 1 0.001 0.001 0.2	0.05 0.005 1 0.001 0.001 0.2

2.2.1.3 Investigating the use of polyhalite as a potassium fertiliser on plants grown in sand with a non-balanced fertiliser regime

Rice (*Oryza sativa cv. Zhenshan 2)* seeds were sown and germinated in sand with deionised water. After two weeks 12 seedlings per treatment were transferred to separate 9 x 9 x 9 cm pots filled with soil composed; sand (10%), vermiculite (15%) and John Innes 2 (75%). Pots were placed in 14 cm diameter, 4 cm high plastic pots covered in black tape to reduce evaporation. Plants were fed after transfer and then twice more at 3 week intervals after transfer to pots and were arranged in a complete randomised block. A total of four fertiliser treatments (table 2.4) were used 1) a control of an adjusted yoshida nutrient solution (AY2) 2) KCl granules 3) K_2SO_4 granules 4) polyhalite granules (PG) with treatments 2, 3 and 4 having additional N, P and micronutrients added in solution at each fertilisation timepoint. One complete set of plants for the four treatments was grown to allow for destructive harvesting one month after the first panicle had fully emerged, resulting in a total of 48 plants. This block of 48 plants was replicated 3 times at different times of the year to reduce any seasonal effects on results.

Table 2.4 – Nutrient concentrations for experiment 2.2.1.3 and 3.2.1.1

Nutrient	Chemical providing nutrient	Concentration of nutrient in Poly Granules medium (mM/l) for pots (PG and PP)	Concentration of nutrient in Adjusted Yoshida medium (mM/l) for pots (AY2)	Concentration of nutrient in K_2SO_4 medium (mM/l) for pots (K_2SO_4)	Concentration of nutrient in KCl medium (mM/L) for pots (KCl)
N	NH_4NO_3	14.5	14.5	14.5	14.5
P	$NaH_2PO_4.2H_2O$	1.5	1.5	1.5	1.5
K	Polyhalite (K_2O) K_2SO_4 KCl	5 - -	- 5 -	- 5 -	- - 5
Ca	Polyhalite (CaO, $CaSO_4$) $CaSO_4$ $CaCl_2.2H_2O$	5.5	- 5 0.5	- - -	- - -
Mg	Polyhalite (MgO, $MgCO_3$) $MgSO_4.7H_2O$	3 -	- 3	- -	- -
S	Polyhalite (SO_3, $CaSO_4$) K_sSO_4 $CaSO_4$ $MgSO_4.7H_2O$	10.5 - - -	- 2.5 5 3	- 2.5 - -	- - - -
Na	Polyhalite (NaCl) $NaH_2PO_4.2H_2O$ Na_2SiO_3 NaCl	1 1.5 1.25 -	- 1.5 1.25 1	1 1.5 1.25 -	- 1.5 1.25 -
Mn	$MnCl_2.4H_2O$	0.05	0.05	0.05	0.05
Mo	$(NH_4)_6.Mo_7O_{24}.4H_2O$	0.005	0.005	0.005	0.005
B	H_3BO_3	1	1	1	1
Zn	$ZnSO_4.7H_2O$	0.001	0.001	0.001	0.001
Cu	$CuSO_4.5H_2O$	0.001	0.001	0.001	0.001
Fe	$FeCl_2.6H_2O$	0.2	0.2	0.2	0.2

2.2.2 General Methods

2.2.2.1 Glasshouse conditions

Plants were grown in a glasshouse in 12 hour day/night cycle. Day temperatures were 28-32 ^{o}C and night temperatures were 24-30 ^{o}C. The relative humidity was maintained between 50 % and 60 %.

2.2.2.2 Growth analyses

All seedlings were weighed before transferral to pots or hydroponics boxes. Harvested plants were split into roots, shoots and panicles (if present), weighed and dried in a fan oven at 80 ^{o}C for 72 hours, after which they were reweighed. The relative growth rate (RGR) of these plants was determined using the following equation: $RGR = (\ln_{w2} - \ln_{w1}) / (t2 - t1)$ where w = weight and t = number of days. Thousand grain weight (TGW) was determined by weighing 50 seeds and multiplying this weight by 20.

2.2.2.3 Measurement of nutrient content

Dried root, shoot and panicle samples were ground into a powder using Ø 5 mm carbon steel balls and a Ball mill (Retsch mm300). A 20 mg sample was ground and digested with 0.5 ml HNO_3 (68 %) for 12 hours at 70 ^{o}C. Digested samples were diluted with 9.5 ml ultra-pure water to have a final concentration less than 5 % HNO_3 and filtered.

Samples were run through a Thermo iCAP 7400 Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES) (Thermo Fisher) to determine concentrations of K, phosphorus (P), calcium (Ca) sulphur (S), magnesium (Mg), sodium (Na), iron (Fe), boron (B), zinc (Zn), manganese (Mn) and copper (Cu). Experiments 2.2.1.2 and 2.2.1.3 measured all the above elements whilst experiment 2.2.1.1 only measured K, Ca, Mg, S, Na, P and Fe.

Carbon (C) and nitrogen (N) content were measured by weighing 50 mg of dry powdered plant parts into tin foil cones of standard weight (OEM 502-186) (Elemental Microanalysis). The foil cones were then crushed to encompass the powdered plant material whilst eliminating as much air as possible. Samples were then run through a Vario Macro Elemental CN analyser (Elementar). This analysis was only performed for experiment 2.2.1.2.

2.2.2.4 Statistical analysis

All statistical analyses were performed using R software (version 3.6.1, R Core Team, 2019). Graphs were produced using the ggplot2 package (Wickham, 2016). Visual inspection of the data led to outliers being removed before statistical analysis. To produce the PCA plots, data was first transformed using the centered log ratio transformation (Greenace, 2019) to account for the compositional nature of the nutrient data. Principal components (PCs) were based on a correlation matrix and calculated using the prcomp. Screeplots were used to visualise the percentage of importance for each PC and the first two PCs were plotted using ggplot2.

In experiment 2.2.1.1, two-tailed t.tests were performed to test the main effects between AY1 and Poly treatments. When data did not fit the assumptions of a t-test, attempts were made to normalise the data through log, logit and square root or cube root transformation of the data. Where attempts to normalise the data failed, Mann-Whitney U tests were used. Significance was set at $p < 0.05$ for all weight and nutrient data

In experiments 2.2.1.2 and 2.2.1.3, ANOVA tests were used to test the main and interactive effects between treatments, followed by a Tukey post hoc test to determine significant interactions when they were apparent. Levene and Shapiro-Wilk tests were used to determine whether the data fitted the assumptions of an ANOVA. When results did not fit the assumptions of an ANOVA, Kruskal-Wallis tests were used to test the main and interactive effects between AY2, PP, PG and PGS treatments, followed by a Dunn's post hoc test to determine significant interactions when they were apparent. Significance was set at $p < 0.05$ for all weight and nutrient data. For further details of statistical tests used see Supplementary information 1.

2.3 Results

2.3.1 Investigating polyhalite as a potassium fertiliser in hydroponically grown plants

2.3.1.1 Growth traits

A hydroponics system was used to compare an adjusted yoshida (AY1) medium, containing soluble salts equivalent to polyhalite (see table 2.2), as a balanced control treatment to determine if polyhalite affected measured variables differently. Yoshida medium

is a nutrient solution containing the nutrients required by plants for healthy growth (Yoshida et al., 1976), in this experiment it was adjusted to match the profile of the nutrients contained in polyhalite.

No significant differences were seen in dry weights of roots, shoots or panicles (Two-tailed t-test and Mann Whitney U test, $p < 0.05$) (fig. 2.1). Similarly, the RGR (appendix fig. 6.1.1) was not significantly different between the treatments at any of the sampling stages (Mann-Whitney U test, $p < 0.05$) except the grain stage where the RGR of polyhalite treated plants was significantly higher than those grown with the AY1 treatment (Mann-Whitney U test, $p=0.02$).

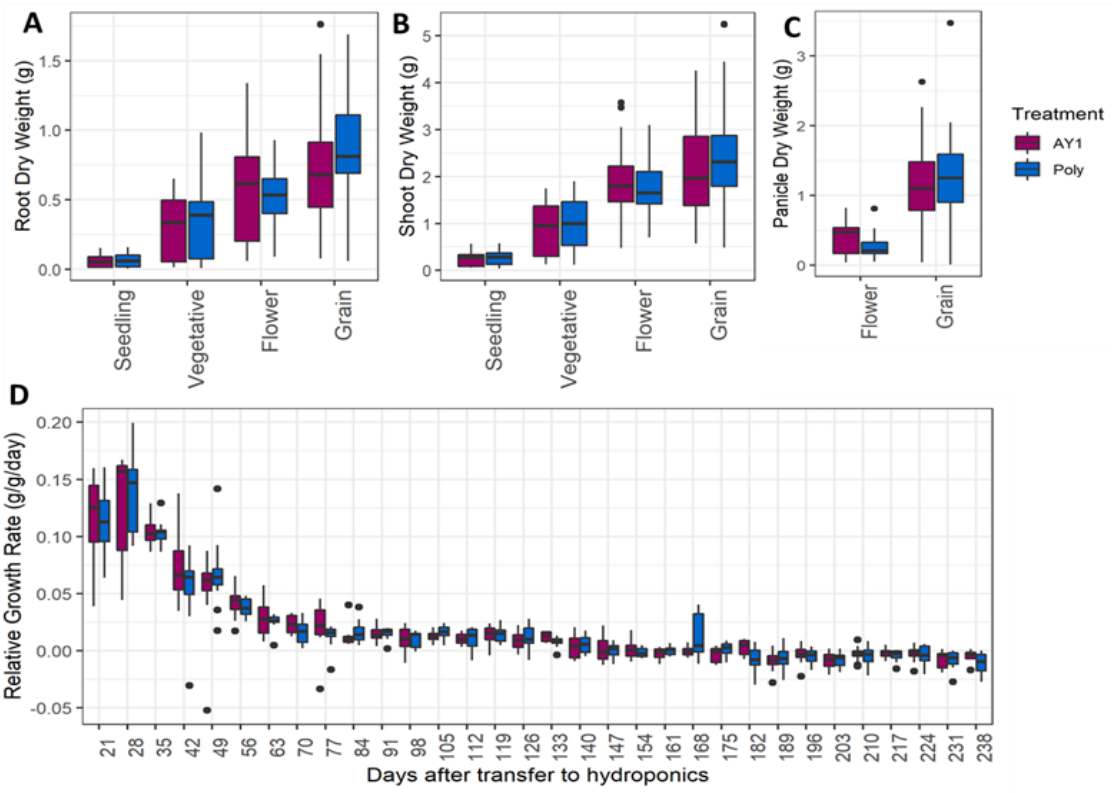


Figure 2.1 – Dry weights of root, shoot and panicles of rice plants grown in hydroponics. Boxplots of dry weights of (A) root, (B) shoot and (C) panicle of plants grown in hydroponics with an AY1 or polyhalite nutrient solution and sampled at four different lifecycle stages. Relative growth rate (D) of plants grown in hydroponics with an AY1 or polyhalite nutrient solution and weighed weekly. No significant differences were determined between treatments ($(p < 0.05)$ Two-tailed t.test or Mann Whitney U test). Lower and upper box boundaries denote the 25th and 75th percentiles, respectively and the central line of the box the median. The lower and upper error denote the minimum and maximum values (calculated as 1.5 x interquartile range), respectively, filled circles represent data falling outside these minimum and maximum values.

2.3.1.2 Nutrient Content

Plants grown in the polyhalite treatment had a significantly higher Mg (Mann Whitney U test, p = 0.015) and Na content (two-tailed t-test, p =0.0019) in their roots at the seedling stage (figs. 2.2 and 2.3), a higher Mg (logit transformed two-tailed t-test, p =0.0002), Ca (Mann Whitney U test, p = 0.0058) and Na (log transformed two-tailed t-test p = 5.5×10^{-5}) content in the roots at the flowering stage and a higher P (two-tailed t-test, p = 0.003) content in the roots at the grain stage, than plants grown in the AY1 treatment. However, the S content (Mann Whitney U test, p = 0.02) of the roots at the vegetative stage in the polyhalite treatment was significantly lower than seen in the AY1 treatment. The Fe content (two-tailed t-test, p = 0.0026) of the shoot was significantly higher in the polyhalite treatment at the seedling stage however this had reversed at the flowering stage with the AY1 treatment having higher shoot Fe content (two-tailed t-test, p = 0.045) at this timepoint. In the panicle at the grain stage, S content (Mann Whitney u, p = 0.0189) was significantly higher in the polyhalite treatment. No significant differences were seen in the K (two-tailed t-test and Mann Whitney U tests $p < 0.05$) content of the plants at any lifecycle stage or plant part (appendix fig. 6.1.2).

Over the lifecycle of the plant, the overall total nutrient content per dry weight (fig. 2.2) remained relatively similar with the only significant differences seen in the roots at the seedling (Mann Whitney U test, p = 0.019), vegetative (log transformed two-tailed t-test, p =0.0095) and flowering (Mann Whitney U test, p = 0.038) stages with the polyhalite treatment having a significantly higher total nutrient root concentration compared to the AY1 treatment at the seedling and flowering stages. However, at the vegetative stage the AY1 treated plants had a higher root total nutrient concentration.

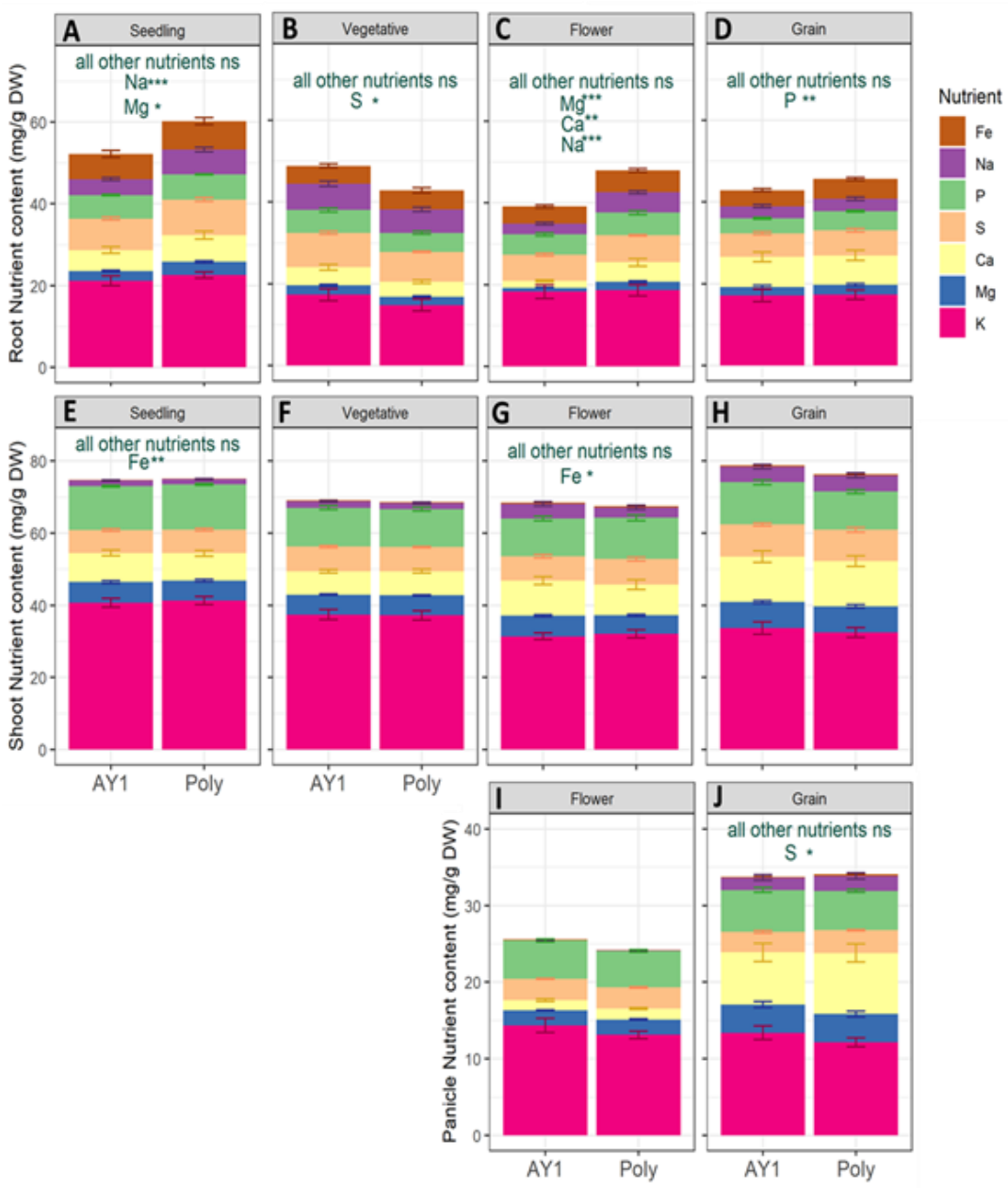


Figure 2.2 – Nutrient content of plants grown hydroponically. Stacked barplots of root (A, B, C, D), shoot (E, F, G, H) and panicle (I and J) nutrient content of plants grown in hydroponics with an adjusted yoshida (AY1) or polyhalite nutrient solution (Poly) and sampled at four different lifecycle stages. Significant differences between treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$, Two-tailed T-test or Mann-Whitney U test.) with labels to denote the nutrient. Each nutrient barplot represents the mean of thirty plants with error bars denoting the standard error.

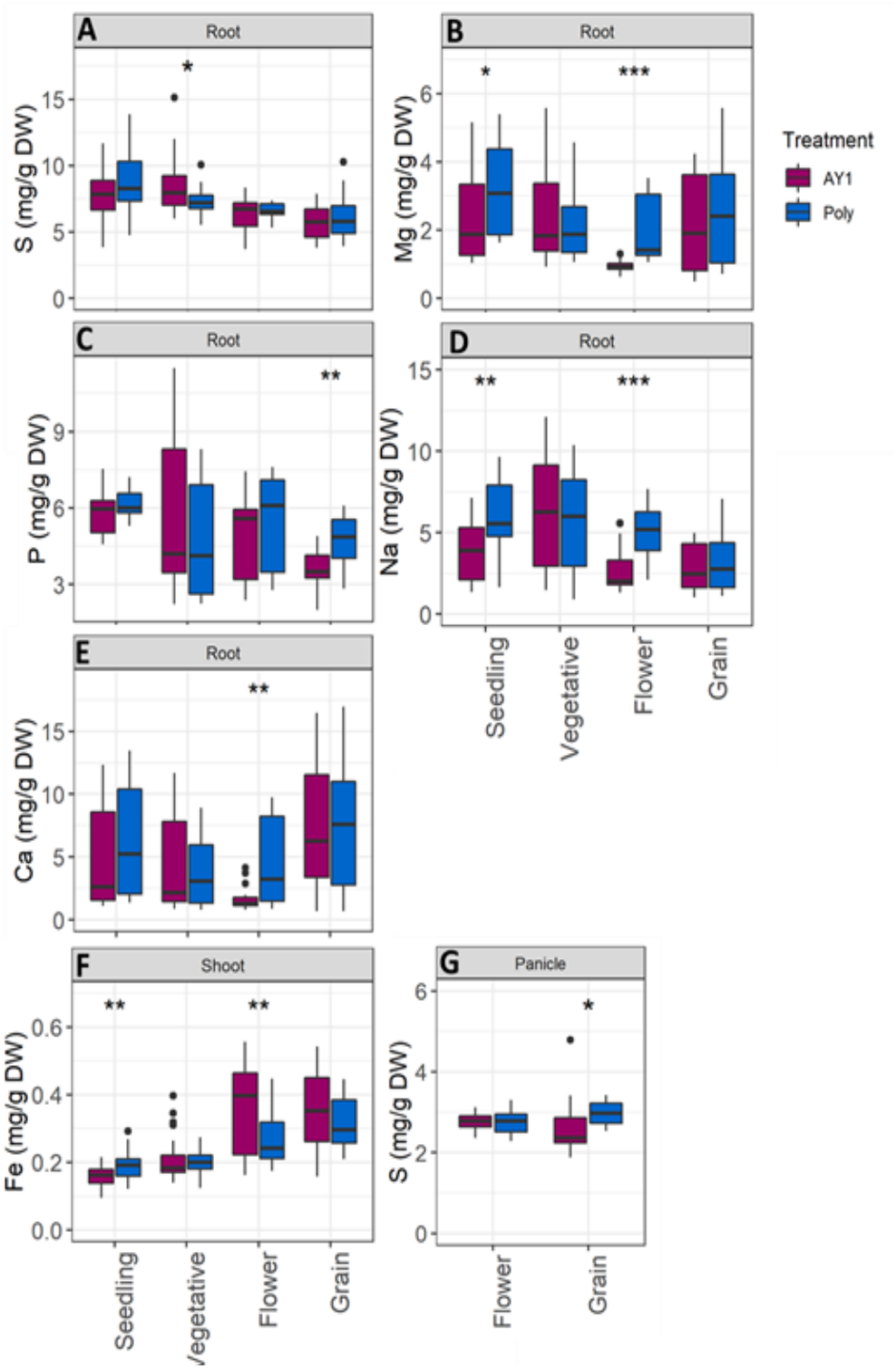


Figure 2.3- Individual nutrient contents with significant differences of plants grown hydroponically. Boxplots of nutrient contents which had significant differences between treatments from plants grown in hydroponics with an adjusted yoshida (AY1) or polyhalite nutrient solution (Poly) and sampled at four different lifecycle stages. See fig. 2.1 caption for explanation of boxplot. Boxplots show root S (A), Mg (B), P (C), Na (D) and Ca (E), shoot Fe (F) and panicle S (G) content. Significant differences between treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ Two-tailed T-test or Mann-Whitney U test). Each boxplot represents thirty plants.

A principal component analysis (PCA) was performed to visualize whether there were any interactions between nutrients or clustering of data points between treatments (fig. 2.4) and to provide a broader picture of any trends that may have occurred with nutrient partitioning between treatments. The first two principal coordinates explained 61.17 % (PC1) and 23.97 % (PC2) of the variance. Loadings of the nutrient variables are marked on the plots with red arrows and are shown in Table 2.5. Clear separation can be seen in the nutrient content between the root, shoot and panicle samples, however the treatment of each sample is relatively mixed between samples. The Fe and Na content of the roots and K, P and Mg content of the shoot and panicles were the driving factors behind PC1 and between the root clustering and the rest of the plant parts sampled. In comparison the S and Ca nutrient contents contributed more to PC2.

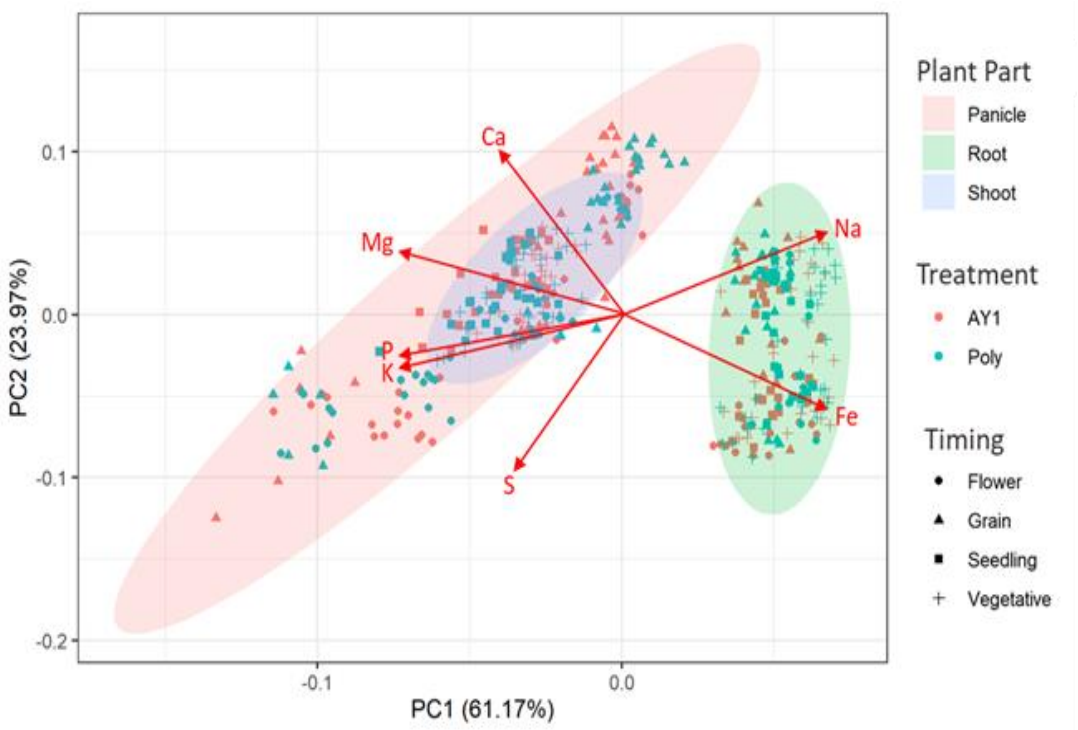


Figure 2.4- PCA of nutrient content of plants grown in hydroponics. Plants were grown with an adjusted yoshida (AY1) or polyhalite nutrient solution (Poly) and sampled at four different lifecycle stages. Each dot represents one root, shoot or panicle of a plant with treatment and sampling time of the sample shown by the colour and shape of the dot. Red arrows demonstrate the nutrient loadings of the PCA. Coloured ellipses contain 95% of the data for the described plant part. Data was transformed by centralised log-ratio prior to performing the PCA to take into account the compositional nature of the nutrient data.

Table 2.5 – Loading data (PC1 and PC2) of nutrients for PCA in figure 2.4. Numbers in bold show the most important contributing nutrients for each PC.

	K	Mg	Ca	Na	P	Fe	S
PC1	**-0.434**	**-0.444**	-0.240	**0.398**	**-0.439**	**0.399**	-0.216
PC2	-0.184	0.233	**0.590**	0.297	-0.153	-0.359	**-0.568**

2.3.2 Investigating polyhalite as a potassium fertiliser in sand grown plants using a balanced fertiliser regime

2.3.2.1 Growth traits

The suitability of using polyhalite as a K fertiliser was investigated using different forms of polyhalite to help determine whether there was any impact on growth, yield or nutrient content when polyhalite is in granular form compared to solution. Granular and powder forms of polyhalite were also used to establish whether the binding agent in the polyhalite granules had any effect on plant growth traits or nutrient content. An adjusted yoshida nutrient solution, containing K_2SO_4, was used as a balanced control treatment to determine if polyhalite affected measured variables differently.

No significant differences were seen in dry weights of roots (vegetative – log transformed ANOVA, flowering and grain – Kruskal Wallis, $p < 0.05$), shoots (ANOVA, $P < 0.05$) or panicles (log transformed ANOVA, $p < 0.05$) (fig. 2.5). Grain weight and TGW also showed no significant differences between treatments (appendix fig. 6.1.3).

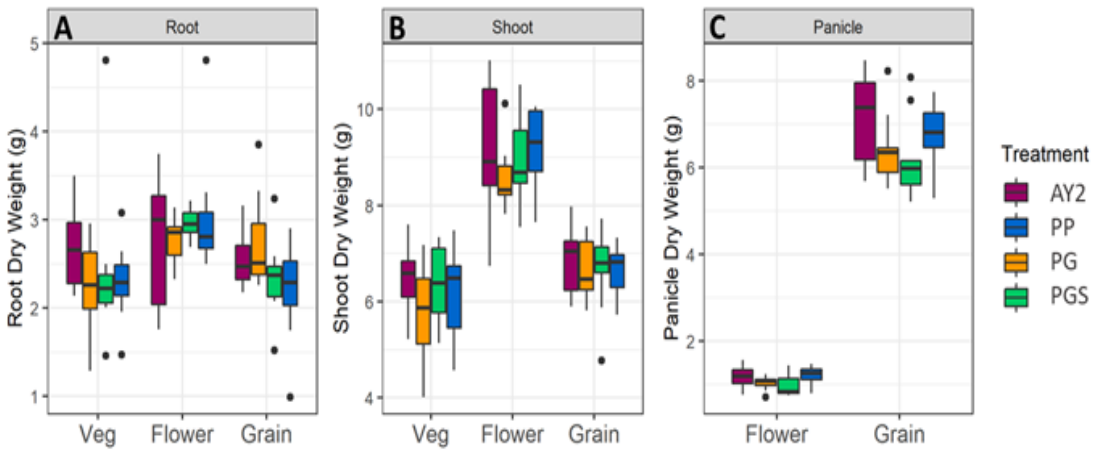


Figure 2.5 Dry weights of root, shoot and panicles of plants grown in sand with a balanced fertiliser regime. Boxplots of (A) root, (B) shoot and (C) panicle of plants grown in pots with an adjusted yoshida (AY2) nutrient solution, a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG) and sampled at three different lifecycle stages (see fig. 2.1 caption for explanation of boxplot). No significant differences ($p < 0.05$, ANOVA or Kruskal-Wallis) were seen between the treatments in any plant part or at any sampling stage.

2.3.2.2 Nutrient content

Despite the lack of differences in growth and yield, significant differences were seen in the nutrient composition of some tissues for some of the nutrients. Root Fe content was significantly higher in the AY2 treatment than the PGS treatment (Dunn's post-hoc test, $p = 0.012$) at the vegetative stage (figs. 2.6, 2.7 and 2.8) whilst shoot C content was significantly higher in the PGS treatment compared to plants grown with the PG treatment (Tukey's range test, $p = 0.01$). Whilst Fe is by convention classed as a micronutrient, it has been presented in the macronutrients figures due to the high Fe root contents in all treatments which obscured the visualisation of other micronutrients. At the grain sampling stage, plants grown with the AY2 treatment had significantly lower shoot Na content than either the PG (Tukey's range test, $p = 0.03$) or PGS treatment (Tukey's range test, $p = 0.002$). Nutrient contents of K, Mg, P, S, B, N, Cu, Mn, and Zn (figs. 2.6 and 2.7) were unaffected by treatment at all sampling stages (appendix figs. 6.1.4, 6.1.5 and 6.1.6).

Over the lifecycle of the plant, the overall total nutrient content per dry weight as well as the total macronutrient and micronutrient contents per dry weight remained relatively similar with the only significant differences seen in the micronutrient content of the roots (Kruskal Wallis, $p = 0.013$) and shoots (log transformed ANOVA, $p = 0.022$) at the vegetative stage (figs. 2.6 and 2.7). In the roots micronutrient content per dry weight was higher in the PGS treatment than the AY2 treatment (Dunn's post hoc test, $p = 0.0009$), whereas in the shoots plants grown with the PP treatment had a significantly higher micronutrient content per dry weight than those grown in both the PG (Tukey's range test, $p = 0.033$) and PGS treatments (Tukey's range test, $p = 0.038$).

A PCA was performed to visualize whether there were any interactions between nutrients or clustering of data points between treatments (fig. 2.9) The first two principal coordinates explained 37.03 % (PC1) and 25.06 % (PC2) of the variance. Loadings of the nutrient variables are marked on the plots with red arrows and are shown in Table 2.6. Clear separation can be seen between the nutrient content of root, shoot and panicle samples however treatment is relatively mixed between samples. The biggest similarities between data points are within the different plant parts, with the nutrient contents of Fe and Na driving a large proportion of the difference between roots and the rest of the plant in PC1, a factor which can also be seen in fig. 2.6, with the nutrient contents of K, Mg and P being the largest opposite contributors to PC1 (table 2.6). In PC2 the loadings which are having the largest effect are Ca, Cu and C and Mn and B which appear to be the separating factors for the shoot and panicle sample groupings.

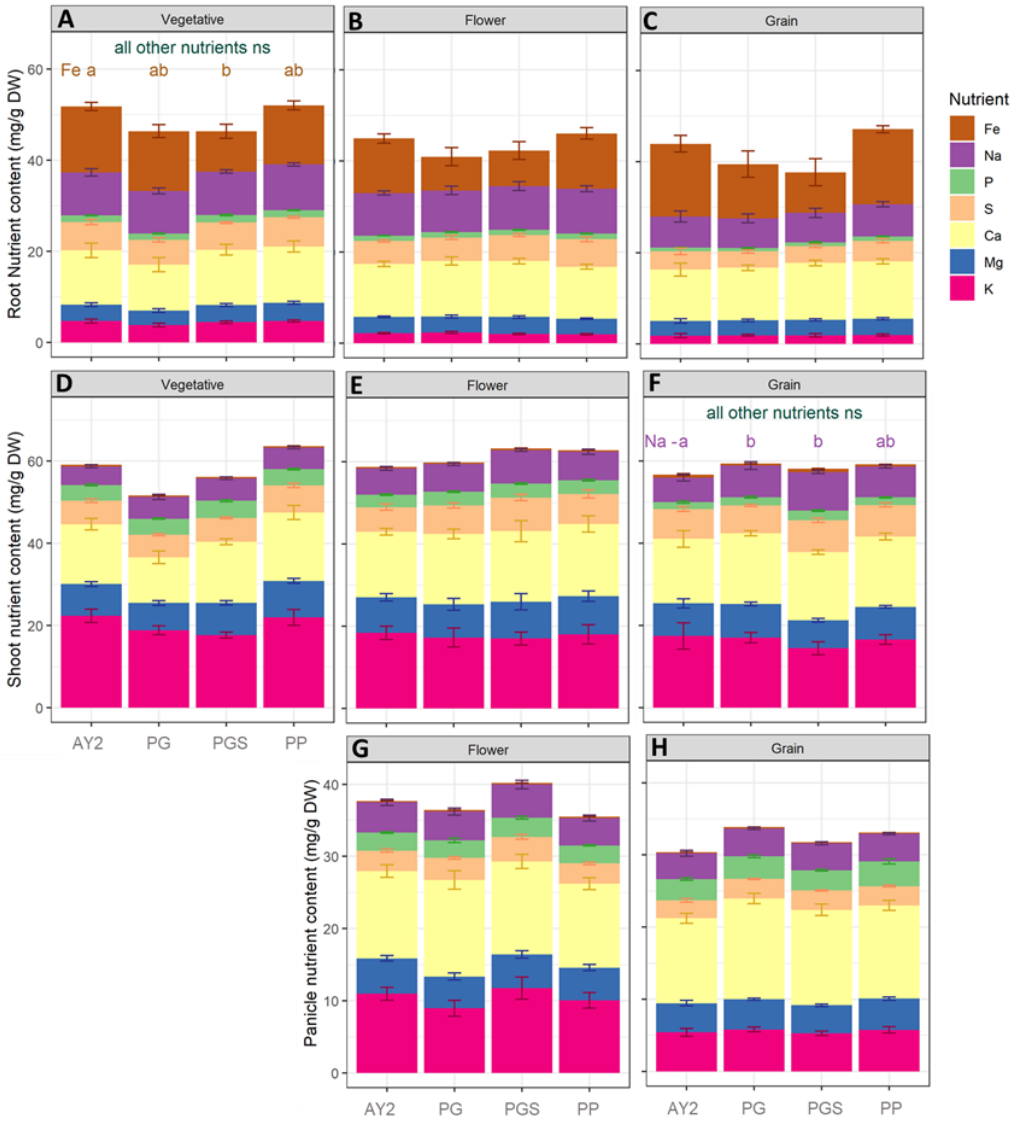


Figure 2.6 – Macronutrient and Fe content of sand grown plants. Stacked barplots of nutrient content of root (A, B, C), shoot (D, E, F) and panicle (G and H) nutrient content of plants grown in pots with an adjusted yoshida (AY2), a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG). Plants were sampled at three different lifecycle stages. Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are denoted with different letters above the barplot with the nutrient they are referring to labelled to the left. Each nutrient barplot represents the mean of nine plants with error bars denoting the standard error.

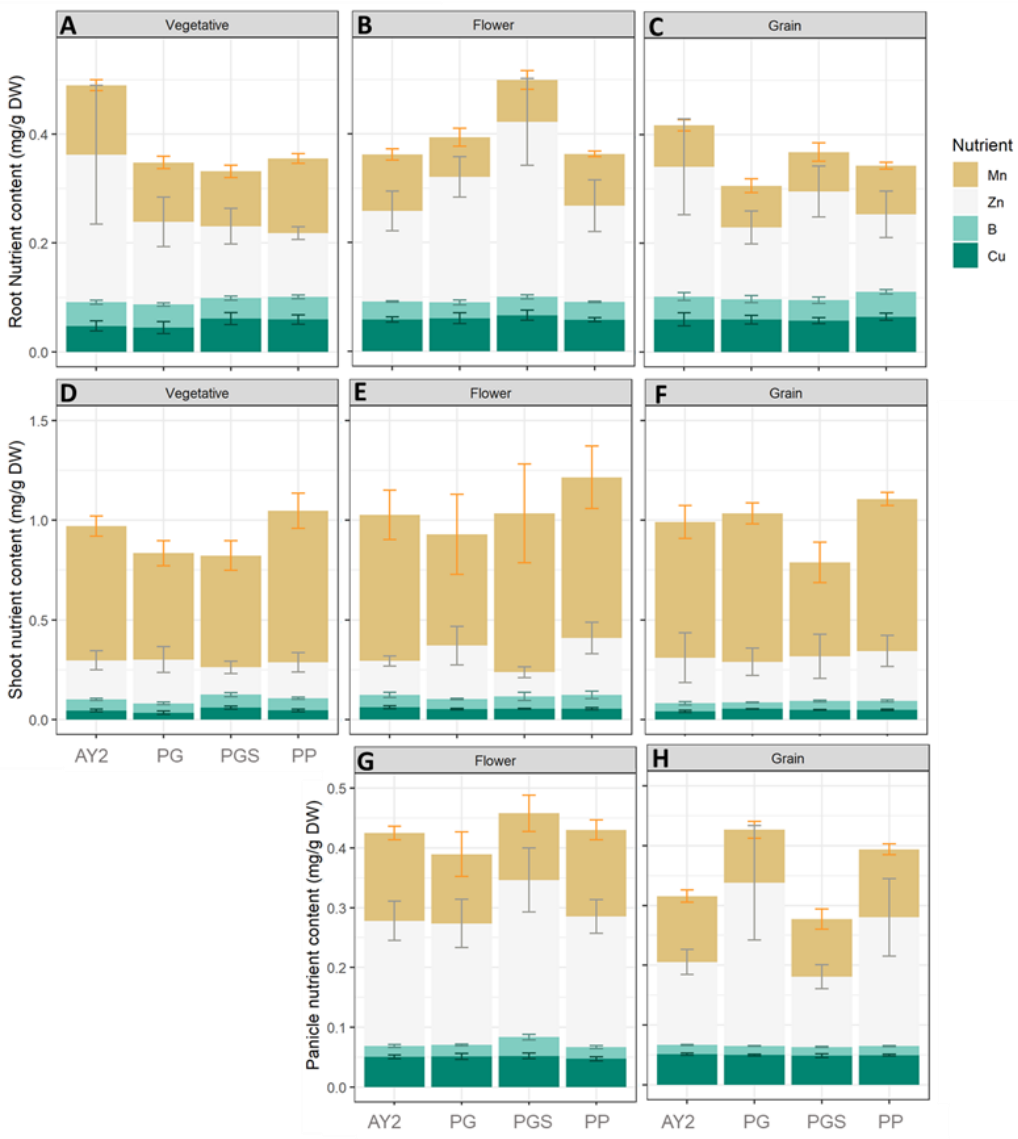


Figure 2.7 – Micronutrient content of sand grown plants. Stacked barplots of nutrient content of root (A, B, C), shoot (D, E, F) and panicle (G and H) nutrient content of plants grown in pots with an adjusted yoshida (AY2), a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG). Plants were sampled at three different lifecycle stages. No significant differences were seen in individual nutrient contents between treatments (ANOVA or Kruskal Wallis, $p < 0.05$). Each nutrient barplot represents the mean of nine plants with error bars denoting the standard error.

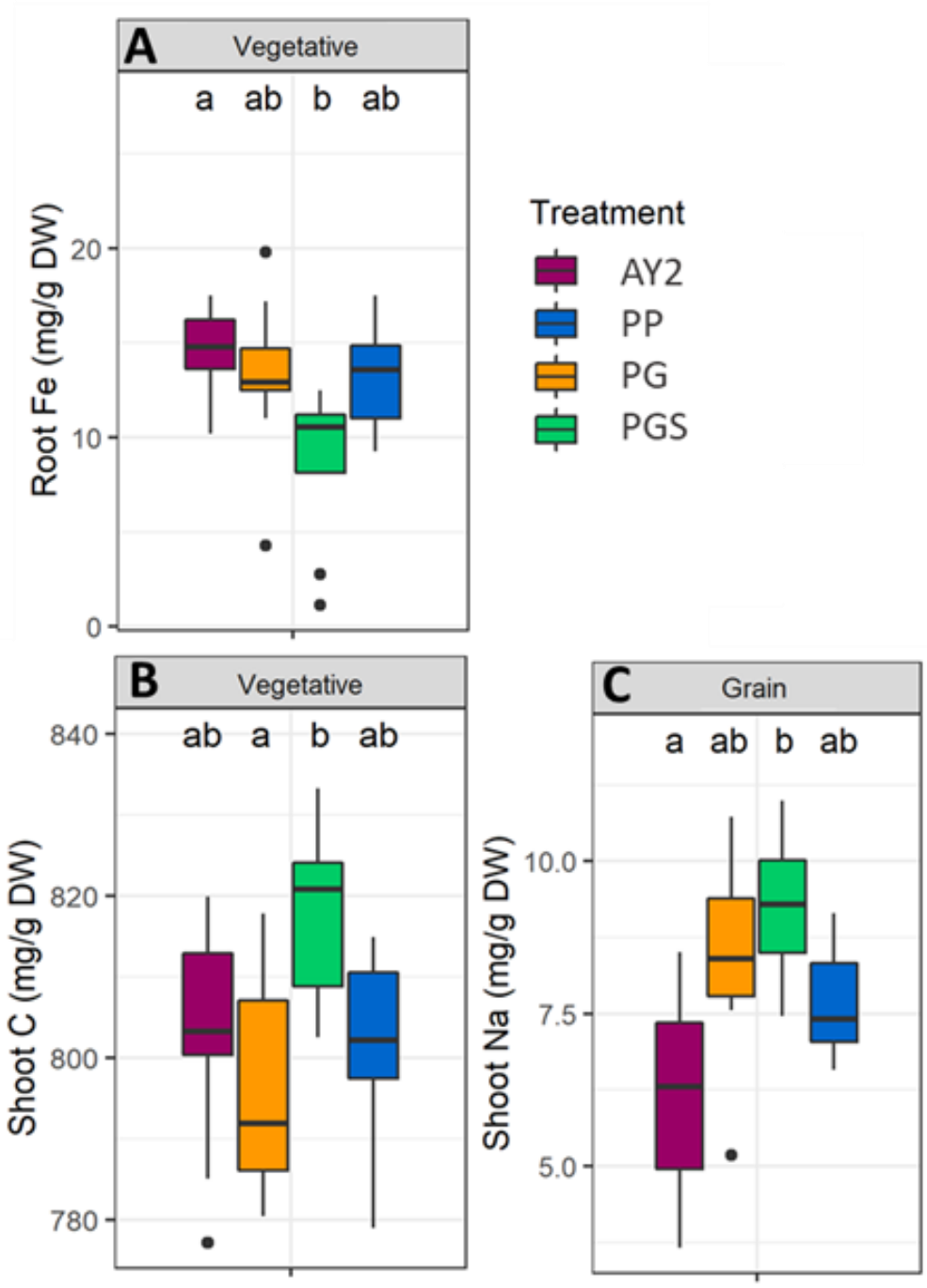


Figure 2.8 – Individual nutrient contents with significant differences of plants grown in sand. Boxplots of root Fe at the vegetative stage (A), shoot C at the vegetative stage (B) and shoot Na at the grain sampling stage (C). These nutrients had significantly difference nutrient content between treatments of plants grown in pots with an adjusted yoshida (AY2), a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG) and sampled at three different lifecycle stages. See fig. 2.1 caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are indicated by different letters above the boxplots. Each boxplot represents nine plants.

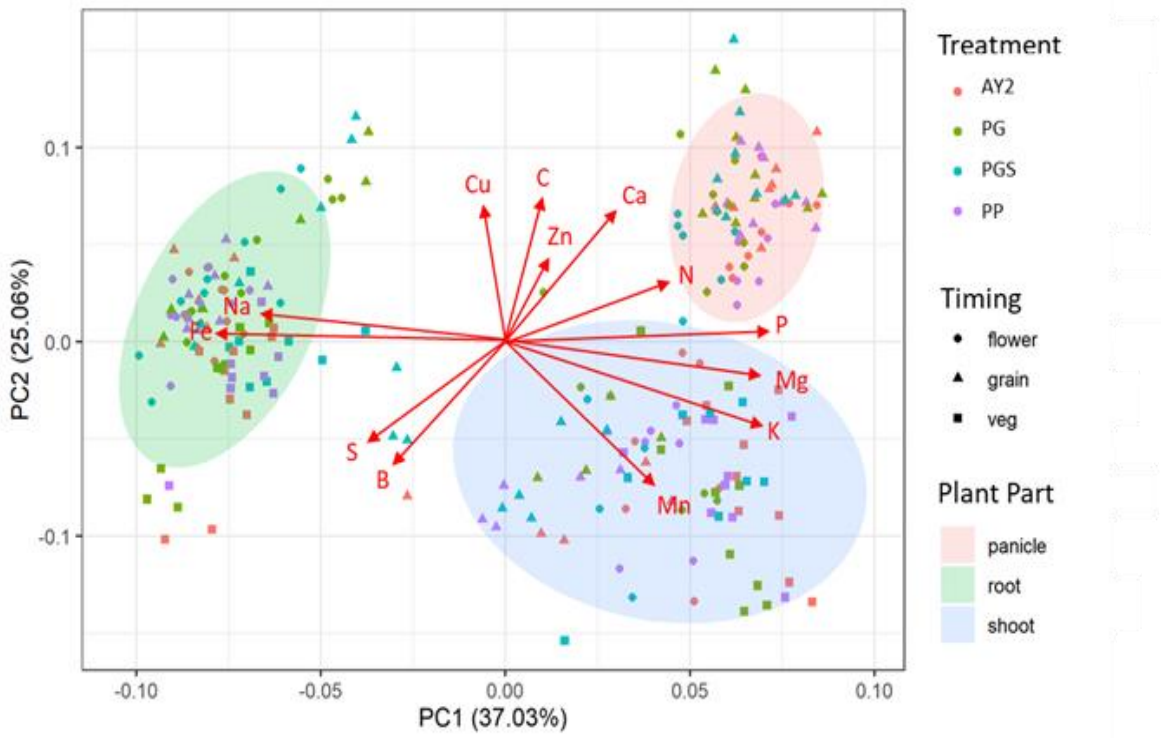


Figure 2.9 - PCA of nutrient content of plants grown in sand with a balanced fertiliser regime. PCA of nutrient data from plants grown in pots with an adjusted yoshida (AY2), a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG). Each dot represents one root, shoot or panicle of a plant with treatment and sampling time of the sample shown by the colour and shape of the dot. Red arrows demonstrate the nutrient loadings of the PCA. Coloured ellipses contain 95% of the data for the described plant part. Data was transformed by centralised log-ratio prior to performing the PCA to take into account the compositional nature of the nutrient data.

Table 2.6 – Loading data (PC1 and PC2) of nutrients for PCA in figure 2.9. Numbers in bold show the most important contributing nutrients for each PC.

	Fe	K	Mg	Ca	Na	Cu	Zn	Mn	B	P	S	N	C
PC1	**-0.439**	**0.387**	**0.378**	0.169	**-0.371**	-0.036	0.063	0.229	-0.167	**0.394**	-0.206	0.247	0.056
PC2	0.025	-0.233	-0.095	**0.375**	0.081	**0.392**	0.240	**-0.414**	**-0.352**	0.035	-0.286	0.175	**0.411**

2.3.3 Investigating polyhalite as a potassium fertiliser in soil grown plants in a non-balanced fertiliser regime

2.3.3.1 Growth traits

The suitability of using polyhalite as a K fertiliser was investigated compared to K_2SO_4 and KCl two of the most widely used K fertilisers. A control (AY2) containing K_2SO_4 and other salts to make a solution equivalent to polyhalite was used, however the other treatments applied only the same rate of K and were not balanced to match the nutrient profile of polyhalite in order to establish whether simply using polyhalite as a substitute K fertiliser would impact on growth, yield or nutrient content of rice. The unbalanced nature of this experiment also provides a more realistic picture of how crops will behave if a farmer were to use polyhalite without changing any other component in their fertiliser regime. No significant differences (ANOVA, $p < 0.05$) were seen in shoot and panicle dry weight (fig. 2.10). Significant differences (Kruskal Wallis, $p = 0.045$) were seen in the thousand grain weight (TGW) with the KCl treatment having a significantly lower TGW than the polyhalite treatment (Dunn's post hoc test, $p = 0.027$) whereas the TGW of plants grown in the AY2 and K_2SO_4 treatments were not significantly different to those in the polyhalite or KCl treatments.

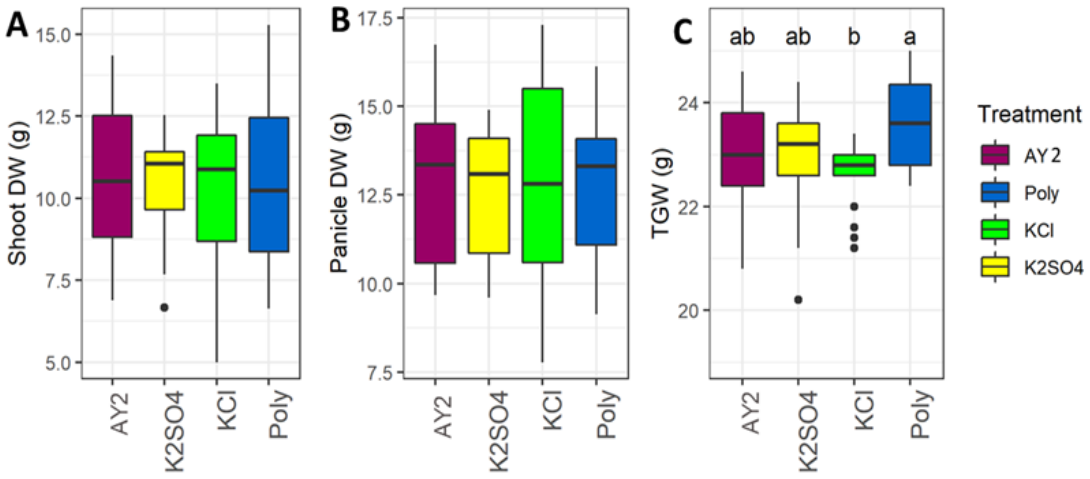


Figure 2.10 - Dry weights of root, shoot and panicles of plants grown in soil with a non-balanced fertiliser regime. Boxplots of dry weights of shoot (A), panicle (B) and TGW (C) of plants grown in pots with an AY2 solution, KCl granules, K_2SO_4 granules or polyhalite granules (Poly) (see fig. 2.1.caption for explanation of boxplot). No significant differences ($p < 0.05$, ANOVA or Kruskal-Wallis) were seen between the treatments the shoot or panicle dry weights, however significant differences were seen in the TGW where different letters above the boxplots denote significant differences between those treatments.

2.3.3.2 Nutrient content

Significant differences were seen in the S content of the shoot (ANOVA, $p = 2.74 \times 10^{-16}$) and panicles (ANOVA, $p = 0.0076$) (figs. 2.11 and 2.12) with the KCl treatment having significantly lower S content in the shoot than all other treatments (Tukey's range test, all $p < 0.001$) and significantly lower S content in the panicle compared to the AY2 treatment (Tukey's range test, $p = 0.02$) and K_2SO_4 treatment (Tukey's range test, $p = 0.009$). In the shoot the P content of the KCl treated plants was lower than that in AY2 treated plants (Tukey's range test, $p = 0.007$) whilst shoot Ca content was significantly higher in the KCl treatment than the AY2 (Tukey's range test, $p = 0.038$) or K_2SO_4 treatments (Tukey's range test, $p = 0.003$). The K content of the panicles was significantly lower in the Poly treatment than the KCl treated plants (Tukey's range test, $p = 0.023$). Significant differences were seen in the Cu content of the panicles (ANOVA, $p = 0.018$) with the KCl treatment having significantly higher Cu content than the AY2 treatment (Tukey's range test, $p = 0.035$). Whilst, panicle Zn content was lower in the KCl treatment than the Poly treatment (Tukey's range test, $p = 0.012$). No significant differences were seen in the root and shoot Mg or Na content between treatments (appendix fig. 6.1.7).

For the overall total, macronutrient total and micronutrient total nutrient contents per dry weight (fig. 2.11), the only significant difference was seen in the shoot micronutrient contents between the Poly and KCl treated plants, where the KCl treated plants had a higher shoot micronutrient concentration compared to the Poly treated plants (Tukey's range test, $p = 0.02$).

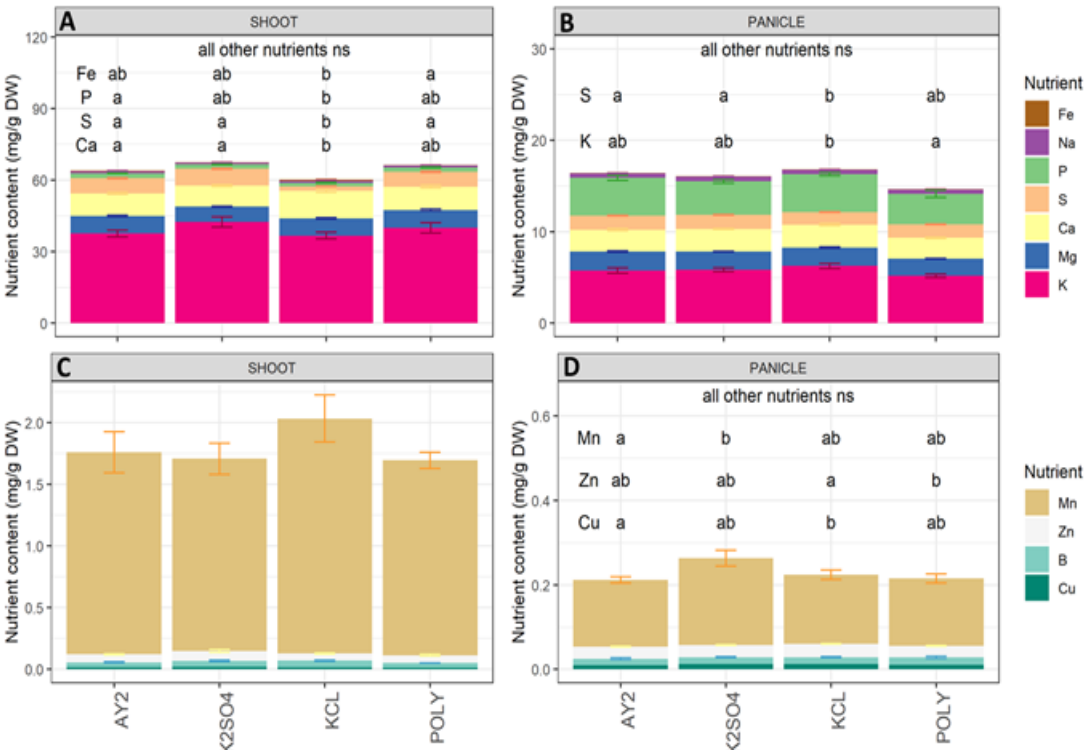


Figure 2.11 – Macronutrient and micronutrient content of plants grown in soil with a non-balanced fertiliser regime. Stacked barplots of shoot (A, B) and panicle (C and D) nutrient content of plants grown of plants grown in pots with an AY2 solution, KCl granules, K_2SO_4 granules or polyhalite granules (Poly). Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are denoted with different letters above the barplot with the nutrient they are referring to labelled to the left. Each nutrient barplot represents the mean of twelve plants with error bars denoting the standard error.

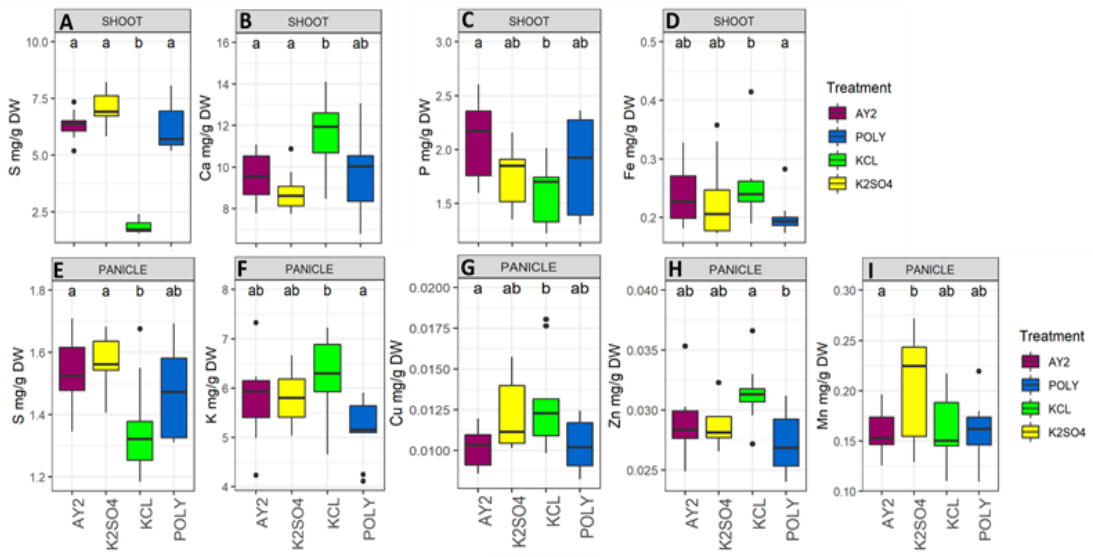


Figure 2.13 – Individual nutrient content with significant differences of plants grown in soil with a non-balanced fertiliser regime. Boxplots of the shoot S (A), Ca (B), P (C), and Fe (D) nutrient contents and panicle S (E), K (F), Cu (G), Zn (H) and Mn (I) nutrient contents. Tissue levels of these nutrients were significantly different between treatments of plants grown in pots with an adjusted yoshida solution (AY2), KCl granules, K_2SO_4 granules or polyhalite granules (Poly). See fig. 2.1 caption for explanation of boxplot. Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are indicated by different letters above the boxplot. Each boxplot represents twelve plants.

A PCA was performed to visualize whether there were any interactions between nutrients or clustering of data points between treatments (fig. 2.13). The first two principal coordinates explained 58.91 % (PC1) and 13.87% (PC2) of the variance. Loadings of the nutrient variables are marked on the plots with red arrows and are shown in Table 2.7. Clear separation can be seen between the shoot and panicle samples however treatment is relatively mixed in the panicle samples. In the shoot sample, the KCl treated plants are clustered separately to the other treatments, with the S content appearing to drive the differences seen between these treatments. The content of P, Na, Cu and Zn and K, Mg, Ca, Fe and Mn content appears to be driving PC1 (table 2.7) and a large proportion of the difference between the shoots and panicles, whereas the nutrient contents of S and B are the most important contributing factors to PC2.

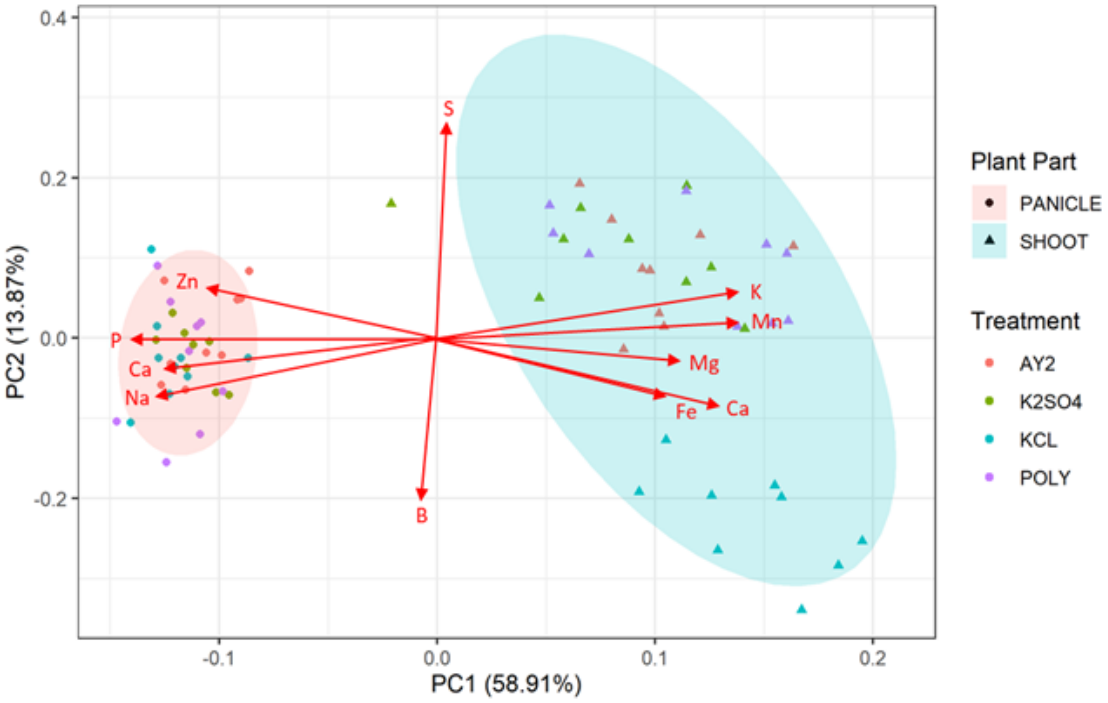


Figure 2.13 – PCA of plants grown in soil with non-balanced fertiliser regime. PCA of nutrient data from plants grown in pots with an AY2 solution, KCl granules, K2SO4 granules or polyhalite granules (Poly). Each dot represents one shoot or panicle of a plant with treatment and plant part of the sample shown by the colour and shape of the dot. Red arrows demonstrate the nutrient loadings of the PCA. Coloured ellipses contain 95% of the data for the described plant part. Data was transformed by centralised log-ratio prior to performing the PCA to take into account the compositional nature of the nutrient data.

Table 2.7 – Loading data (PC1 and PC2) of nutrients for PCA in figure 2.13. Numbers in bold show the most important contributing nutrients for each PC.

	P	S	Fe	K	Mg	Ca	Na	Cu	Zn	Mn	B
PC1	**-0.370**	0.014	**0.281**	**0.367**	**0.298**	**0.344**	**-0.343**	**-0.335**	**-0.284**	**0.361**	-0.022
PC2	-0.003	**0.720**	-0.197	0.152	-0.080	-0.222	-0.203	-0.103	0.164	0.056	**-0.531**

2.4 Discussion

2.4.1 Investigating the use of polyhalite as a potassium fertiliser in hydroponically grown plants.

2.4.1.1 Growth traits

A common shortfall in many polyhalite studies is the lack of a fully balanced fertiliser regime to match the polyhalite nutritional profile (Dal Molin et al., 2020; da Costa Mello et al., 2020; Pavuluri et al., 2017; da Costa Mello et al., 2018a,b; Zhou et al., 2019; Lillywhite et al., 2020). The lack of a fully balanced control can lead to misinterpretation of the properties of polyhalite. Many of the yield increases reported in the literature (Pavuluri et al., 2017; Zhou et al., 2019; da Costa Mello et al., 2018a, 2020, 2018b; Lillywhite et al., 2020) are more likely due to the additional nutritional supply from polyhalite, compared to a KCl or K_2SO_4 treatment. For example, Pavuluri et al (2017) reported an increased yield in maize plants grown in Tanzanian field conditions when using polyhalite compared to KCl or K_2SO_4 based fertilisers. However, no yield difference was seen when comparing polyhalite to a treatment containing KCl and kieserite, a compound containing K, S and Mg. Whilst the KCl and kieserite treatment did not fully match the polyhalite nutrient supply, the lack of significant yield difference between these treatments suggests that the reported increases in yield for polyhalite compared to KCl and K_2SO_4 were due to the additional Mg and/or Ca supplied. Results in barley comparing polyhalite, KCl and K_2SO_4 found that KCl treated plants had a lower yield than those in polyhalite or K_2SO_4 treated plants (Lillywhite et al., 2020) suggesting that the S content of polyhalite and K_2SO_4 may have impacted the yield. In comparison, the results from the fully balanced hydroponics experiment described here show no difference in the dry weights of roots, shoots or panicles between treatments (fig. 2.1). Significant differences were seen in the RGR of polyhalite treated plants, but only at the latest lifecycle sampling point, suggesting treatment type did not have an overall effect on RGR throughout the plant lifecycle (see appendix fig. 6.1.1).

2.4.1.2 Nutrient content

The nutritional content of crops is important not only for healthy plant growth which can affect crop yields, but also in terms of the end product providing nutrition for humans. As most other experiments using polyhalite have been field based, there are little to no data in

the literature regarding root nutrient contents. Additionally, studies thus far have only taken one timepoint for plant sampling with most focusing on crop harvest as their measurement timepoint. Therefore, it is difficult to interpret these results compared to the literature. Nevertheless, in the hydroponics experiment no significant differences were seen in the K content of any plant part at any sampling stage (fig. 2.2 and appendix fig. 6.1.2) which is similar to results found in maize (Dal Molin et al., 2020) and tea (Zhou et al., 2019).

Foliar increases in S have been seen in polyhalite treated tomatoes compared to KCl treatments (da Mello et al 2020). Whilst no difference was seen in shoot S in this experiment (fig. 2.2 and appendix fig 6.1.2), increased S content was seen in roots under the AY1 treatment at the vegetative stage and higher S content in the grain under the polyhalite treatment at the panicle stage. Due to differences in crop species contents of seed S, with members of the Brassicaceae and Leguminosae families containing higher quantities than members of the Poaceae family (Marschner, 2012), the increased grain S content in the polyhalite treatment may be beneficial in crops from these families.

Increases in root Mg content at the seedling and flowering stages of hydroponically grown plants provided with polyhalite were observed (figs. 2.2 and 2.3). In root cells, Mg is required for a number of biochemical processes including nucleic acid synthesis, enzyme activation and carbohydrate metabolism (Niu et al., 2014). The increased root Mg observed in the polyhalite treatment may have temporarily boosted these activities, although it did not significantly affect plant dry weights. Additionally, these increases in root Mg were not transferred to shoot Mg content as no significant differences in shoot Mg were seen between treatments. This was contrary to the foliar increases seen in tea, where polyhalite treated plants contained significantly more Mg (Zhou et al., 2019). Similarly da Costa Mello et al (2018b) found that tomatoes provided with fertiliser regimes containing additional S, (K_2SO_4, $K_2SO_4 \cdot 2MgSO_4$ and polyhalite) had higher foliar Mg.

Increased root Ca content in the polyhalite treatment at the flowering stage was seen in this experiment, however no significant differences in shoot Ca content were found between treatments. In contrast, increased foliar Ca has been reported in tea grown with polyhalite (Zhou et al., 2019). Furthermore, mixed results were obtained in tomato where

shoot Ca content was increased after polyhalite treatment in one study (da Mello et al 2020) whilst in another study the same authors found higher foliar Ca in response to a fertiliser regime which did not include any K fertiliser (da Mello et al 2018a).

Soil Mg and Ca deficiencies are rare in irrigated rice fields as an adequate supply is usually proved in the irrigation water, although lowland and upland rice fields are more frequently depleted of Mg (Dobermann & Fairhurst, 2000). It would be of interest therefore to observe whether plants grown in Mg deficient soils are given an advantage when provided with polyhalite as a K fertiliser due to the increase in root Mg seen in this experiment. The reduction of Fe compounds after paddy field flooding can lead to displacement of soil associated Mg and Ca ions due to increased Fe ions within the soil (Dobermann & Fairhurst, 2000). Therefore, further work investigating polyhalite in paddy field settings would be beneficial to help evaluate its effect with these additional factors.

As well as containing the four macronutrients: K, S, Mg and Ca, polyhalite also contains trace quantities of the micronutrients: B, Zn, Mn, Mo, Fe and Cu. The increase of Fe seen in the shoot at the seedling stage for the polyhalite treatment of the hydroponics experiment could be due to the trace quantities of Fe found within polyhalite, however it is unlikely. Polyhalite is a natural mineral therefore the trace quantities of micronutrients it contains may have some variability, however the quantity of Fe is generally < 0.5 mg kg^{-1} (Barbier et al 2017). Therefore, the concentration of Fe added through polyhalite was very minimal, being approximately 10000 times lower than that typically found in nutrient solutions. Furthermore the slight increase in shoot Fe of AY1 grown plants at the later flowering stage was not conveyed to the panicle where it could have acted as a beneficial improvement due to the importance of Fe in the human diet, especially as Fe deficiency is common both in plant and human populations (Sharma et al., 2013).

At low and moderate concentrations Na is often classed as a beneficial nutrient (Barker & Pilbeam, 2007) and can partially act as a substitute for K when it is deficient (Hartley et al., 2020). However, in high concentrations Na can be toxic to plant growth and salinization poses challenging conditions in agricultural settings. Sodium has been shown to stimulate growth in a variety of crops even with sufficient K present (Subbarao et al., 2003) and

therefore the increased root Na content in the polyhalite treatment at the seedling and flowering stage seen in this study may be beneficial in a field setting.

Over half of global agricultural land is reported to be P deficient (Cakmak, 2002). This global lack of available soil P is combined with diminishing supplies of P rock reserves. In this study the increased root P content was evident in the polyhalite treatment but only at the grain stage. Increased root P may be beneficial to the plant. However as plants have their highest P requirement at early growth stages (Römer & Schilling, 1986), an increased root P content at the grain stage is less likely to have a considerable effect on a crop.

The differences between the treatments' average overall nutrient content in the roots could be seen at different lifecycle stages, however the treatment containing the highest average overall nutrient content varied throughout the plant lifecycle. Principal component analysis showed a greater emphasis of nutrient partitioning by plant part and the absence of clustering between treatments when comparing the overall nutrient composition of plants provided with different K fertiliser regimes is promising for the use of polyhalite as a K fertiliser.

2.4.2 Investigating the use of polyhalite as a potassium fertiliser in sand grown plants with a balanced fertiliser regime

2.4.2.1 Growth traits

As most rice crops are grown in a field setting, it was important to compare the growth of rice plants provided with a balanced fertiliser regime in a more realistic growth setting. Plants were grown in sand, to avoid additional nutrition from the growth substrate, therefore reducing some of the confounding factors of additional nutrition from this source, as well as avoiding soil nutrient interactions from the growth substrate. Additionally, nutrient inputs were kept low to help evaluate whether polyhalite had a beneficial effect in a non-saturated nutrient environment. Another important factor to compare was the form of polyhalite applied to the crop. One of the main forms in which polyhalite will be commercialised is as granules approximately Ø 2-4 mm which contain polyhalite powder bound with corn starch (Lewis et al., 2019). These granules allow for the slow release of the nutrients contained in polyhalite. This experiment was designed to observe whether the slow-release of these

granules had any beneficial effect on plant growth and nutrition. Dissolved polyhalite granules were used as a comparison with polyhalite powder to consider whether the binding agent contained in the granules had any effect on the plant. The use of the undissolved polyhalite granules allowed comparison between the slow-release nature of the granules and an immediate release of nutrients.

In the balanced pot experiment, no significant differences were seen in root, shoot or panicle dry weights at any of the sampling stages (fig. 2.5). In contrast to studies discussed above, da Costa Mello et al., (2018a) came closer to balancing fertiliser regimes through the use of K_2SO_4, kieserite (containing K, S and Mg) and gypsum (containing Ca and S), in two field experiments on potatoes. They found that yield was higher in the polyhalite and K_2SO_4 + kieserite treatments compared to the KCl treatments at one location which may be due to the additional nutrients delivered by K_2SO_4 + kieserite. These yield increases were not seen in their second location, demonstrating the importance of testing different fertiliser treatments in multiple locations that may vary in soil nutrient content as polyhalite may be more beneficial on certain soil types.

2.4.2.2 Nutrient content

Whilst this experiment showed no significant difference in K content in any plant part at any sampling stage (figs. 2.6 and 2.8), da Costa Mello et al. (2018a) had inconsistent results in tomato plants with results from one location having no significant differences whereas those from another location showed higher foliar K in the polyhalite treatment compared to their KCl and K_2SO_4 treatments. In addition, higher fruit K content was found in their polyhalite treated tomatoes compared to all other treatments. The release of K from different polyhalite forms has been reported to be highest from granules followed by powder (Lewis et al., 2019), however this did not affect plant K content in this study. Whilst a low fertiliser rate was used to grow the plants it may still have provided sufficient or even saturated K conditions therefore reducing differentiation between the fertiliser forms. A further experiment with multiple K rates would help to address this issue.

In experiments looking at a slow releasing KCl fertiliser product, Tian et al., 2017 found that cotton yields were significantly increased when provided with a slow release

fertiliser compared to conventional KCl or K_2SO_4 fertiliser products. Their study only provided a single application of K fertiliser, whereas in the hydroponics experiment, the nutrient solution was changed weekly and in the pot treatments, fertiliser was applied in 4 split applications in an effort to replicate farmer application practices more closely (Dobermann & Fairhurst, 2000). Applying K fertilisers in split applications has been seen to aid in soybean growth and yield (Kolar & Grewal, 1994). Whilst fertiliser addition was kept below the levels usually applied by farmers, it may be that the split application allowed the nutrients from the adjusted yoshida treatment to be available for longer than if they were applied in only a basal application.

In accordance with results seen in maize (Dal Molin et al., 2020) and tea (Zhou et al., 2019), the balanced pot experiment showed no significant difference in S content at any stage. Similarly this experiment did not find any significant difference in Mg content in root, shoot or panicle at any sampling stage which corresponds with results seen by da Costa Mello et al (2020) in tomatoes. The Ca content in this balanced study did not differ significantly between treatments which parallels results found in a maize (Dal Molin et al., 2020).

The slow release nature of the polyhalite granules appears to have made no noticeable effect on the K, S, Ca or Mg content of the plants, suggesting that in these settings it has negligible benefit. In a field setting this feature may help reduce losses through leaching and therefore further trials are necessary.

The lower levels of Fe seen in the roots of PGS plants compared to AY2 treated plants may be beneficial in field settings where iron toxicity is often a problem. Iron toxicity occurs when there are large concentrations of Fe in the soil leading to excessive Fe uptake which can lead to overproduction of ROS and subsequent structural damage (Schmidt et al., 2020).

Increased shoot Na content in the PGS treatment compared to the AY2 treatment at the grain stage did not have any effect on panicle dry weight suggesting that whilst Na content is increased it is not having an effect on crop yield.

Corn starch, the binding agent of polyhalite granules is a potential source of carbon and therefore may have led to an increase in the shoot C content at the vegetative stage seen in the PGS treatment compared to the PG. Dissolving the granules for the PGS treatment may

have provided accessible sugars for the plants to take up directly or an influx of easily available C to the soil which may have increased microbial community populations. In either case the increase may have temporarily benefitted the shoot growth in these plants which, despite not being statistically significant, can be observed as a trend in in fig. 2.5. This influx of easily available C from polyhalite granules may have further ramifications for soil microbial communities and nutrient interactions which may have undesirable effects for the crop. Alternative binding agents may be available which may not affect microbial communities, for example a variety of substances are used in creating a matrix and/or coating slow release fertilisers range including inorganic substances such as clays, organic natural polymers such as vegetable gum or lignin, and synthetic polymers such as resins or polylactic acid (Fu et al., 2018) . However, these would need to be studied further to ensure their viability in the field and to ensure they did not incur other problems such as pollution.

The inclusion of a higher number of nutrients, compared to the results in section 2.3.2.1, did not produce any clustering between treatments when using PCA to compare the overall nutrient composition of the plants (fig. 2.9). As in the hydroponics experiment the overall nutrient content was more strongly influenced by plant part than either the harvesting timepoint or treatment type. Whilst there were changes to the content of individual nutrients, these did not create changes to the overall nutrient pattern. As both this experiment and the experiment in 2.3.2.1 contained balanced fertiliser regimes, investigating the direct comparison of K fertilisers without balancing all nutrients was also important to help evaluate the effect of the K fertilisers individually on overall and single nutrient contents.

2.4.3 Investigating the use of polyhalite as a potassium fertiliser in soil grown plants with a non-balanced fertiliser regime

2.4.2.1 Growth Traits

Whilst the inclusion of balanced fertiliser regimes is important for assessing the efficacy of polyhalite as a K fertiliser, it is also important to compare K fertilisers in a non-balanced approach, more representative of real-world settings. Therefore, this experiment was used to compare polyhalite with the most popular K fertilisers on the market, KCl and K_2SO_4 along with an adjusted yoshida nutrient solution matched to the polyhalite nutrient profile. The K fertilisers were added in such a way as to equalise K inputs across treatments but

not add additional S, Ca or Mg to make them equivalent to polyhalite. Plants were grown in soil adding more complexity to the growth substrate, both through additional nutrition from the soil and also due to nutrient interactions within the soil. Whilst the results in this study showed no difference in root, shoot or panicle dry weights, an increase in TGW in the polyhalite treatment compared to the KCl treatment was determined which could constitute yield gains in the field (fig. 2.10). The observed increase in TGW in the polyhalite treatment was similar to those reported in maize (Pavuluri et al 2017), tomato (da Costa Mello et al 2018a) and potato (da Costa Mello et al 2018a), therefore further field testing using rice would be beneficial.

2.4.3.2 Nutrient content

Despite similar K supply in all fertiliser treatments, panicle K content was higher in the KCl treatment than the polyhalite treatment although no differences were seen in the shoot K content (figs. 2.11 and 2.12). This result is contrary to unbalanced studies in tomatoes, where foliar leaf K was higher in polyhalite treatments compared to KCl treatments (da Costa Mello et al., 2020) and fruit K content was higher in polyhalite treatments compared to KCl, K_2SO_4 and $K_2SO_4 \cdot 2MgSO_4$ treated plants at one location (da Costa Mello et al., 2018b).

Regardless of the K fertiliser source, the predominant mode of supply of K to plant roots occurs through diffusion, although a small quantity is acquired through transpiration-driven mass flow (Syers, 1998). Potassium ions are absorbed into the root by the epidermal and hypodermal cells (Ahmad & Maathuis, 2014). Therefore, the higher K content in the rice panicles of the KCl treated plants was unexpected as all treatments were provided with the same rate of K application.

The decreased shoot S content of the plants treated with KCl compared to all other treatments and lower panicle S content in the KCl treatment than in the K_2SO_4 and AY2 treatments is similar to results found in the foliage of tomato plants (da Costa Mello et al., 2020), however an accompanying increase was not also seen in the fruits. The decreased S in the shoots and panicles of KCl treated plants was expected due to the absence of applied S from the KCl treatment, whereas all other treatments contained S (fig 2.13). The lowered S content in the KCl treatment also influenced the overall nutrient composition in the shoot as

the samples of this treatment were clustered separately to all other treatments in the PCA (fig. 2.14). Additionally, the lower K in the panicles of the polyhalite plants compared to the KCl treated plants suggests that the increased TGW in the polyhalite treatments is unlikely to be associated with K, whereas the alterations in S content may play a role in these changes. Due to the importance of S in protein synthesis and the many roles of S containing secondary metabolites this reduction in S content could have detrimental effects in agricultural settings. Global agricultural S deficiencies are becoming more widespread, with predictions suggesting they are likely to become more frequent and severe if agricultural practices do not change (Feinberg et al., 2021). Therefore, in the future the use of an S containing K fertiliser may be effective in mitigating this problem.

The K content of polyhalite and K_2SO_4 are very different with the former containing 11.6 % K and the later containing 41.5 % K, whereas the S contents are very similar with 19.2 % in polyhalite and 18 % in K_2SO_4. Therefore, when supplying the same rate of K from these two different fertilisers the S content is notably different, with nearly 4 times as much S applied with the polyhalite treatment. Despite the increase in S supply in the polyhalite treatment, the highest S shoot content was found in the K_2SO_4 treatment (figs 2.12 and 2.13). Results in barley and maize also showed little difference in S content between polyhalite and K_2SO_4 treatments (Lillywhite et al., 2020) suggesting that whilst polyhalite supplies more S it is not utilised by the crop, perhaps due to the application being in excess of plant requirements. High quantities of soil S are not generally a problem for plant growth, however it can act antagonistically with B and Mo uptake (Barker & Pilbeam, 2007). Additionally Ca deficient soils and soils with a low buffering capacity can suffer Mg and Ca losses when sulphate ions are in excess (Lillywhite et al., 2020). Consideration of soil nutrient status prior to crop planting should therefore be taken to ensure that excess S does not cause problems. Utilising a blend of K fertilisers e.g. polyhalite and KCl together, may allow for more targeted applications of the nutrients required by a crop.

Shoot Ca content was higher in the KCl treatment compared to the AY2 and K_2SO_4 whereas in previous studies in tea (Zhou et al., 2019) and tomato (da Costa Mello et al., 2020) plants with a polyhalite treatment had higher Ca foliage content than KCl or K_2SO_4 treatments. In irrigated rice fields, Ca deficiencies are rare due to sufficient soil supplies as well as additional Ca being present in irrigation water (Dobermann & Fairhurst, 2000). Overuse of K

and Mg fertilisers can lead to Ca deficiencies due to high levels of soil K and Mg reducing uptake of Ca (Marschner, 2012), however the quantities of these nutrients within polyhalite did not impair Ca uptake.

Iron is involved in the biosynthesis of two major protein groups (Barker & Pilbeam, 2007) and therefore is essential for plant health, however in high concentrations can lead to toxicity. Iron toxicity in rice plants is most severe at early growth stages and can lead to stunted growth and damaged root systems (Dobermann & Fairhurst, 2000). Higher shoot Fe content was seen in the KCl treated plants compared to the polyhalite treated plants. No signs of toxicity were recorded alongside the increased Fe content in this treatment, however in fields with histories of Fe toxicity, using polyhalite may be beneficial compared to KCl.

Panicle Cu and Zn content were also seen in the KCl treatment compared to the AY2 and polyhalite treatments respectively. Zn deficiency is the most widespread micronutrient deficiency and causes problems both in plant and human health (Rudani et al., 2018). A relationship between S deficiency and increased Zn has been noted in Chinese cabbage (Reich et al., 2016) which may be related to the increased Zn found in the KCl treated plants. However, the lower Zn in the panicles of polyhalite treated plants compared to KCl treated plants remains a concern and future polyhalite studies should consider including Zn content measurements.

2.4.4 Overall discussion and conclusions

In terms of rice growth, polyhalite does not appear to have any particular advantage nor disadvantage over other K fertilisers in either balanced or unbalanced situations. The slow release nature of the polyhalite granules does not appear to convey any benefits in terms of biomass production and/or yield. Nonetheless, the slow release nature of polyhalite granules may still be valuable for farmers, who can apply all their K fertiliser at one timepoint, thus reducing labour costs and providing farmers with more time, a valuable commodity in any business but especially so in farming where seasonal restrictions add additional constrictions on farmers time. A reduction in the number of K fertiliser applications needed may also reduce machinery usage which may lower a farms carbon footprint as well as preventing soil compaction from repeated tractor movements. In addition, polyhalite granules may reduce

leaching of K and other nutrients from the soil. Further experiments comparing balanced and unbalanced fertiliser regimes in a field setting would give more clarity as to whether polyhalite generates increased crop yield. The use of a controlled environment can reduce the confounding factors found in a field setting, helping to establish how polyhalite performs compared to other K fertilisers in a balanced setting. However, differences seen within a glasshouse setting may well differ to those seen in the field, therefore further experimentation in a field setting may help to increase our knowledge in this area.

Inconsistent results were seen in nutrient content between the different growing substrates, irrespective of whether balanced or unbalanced fertiliser regimes were applied. For example, in hydroponics S content was higher in the roots at the vegetative stage, however, significant differences in root S content were not seen in either pot experiment. These inconsistent nutrient results reflect those found in the literature, where a variety of crop species showed differing nutrient results (Pavuluri et al., 2017; Zhou et al., 2019; da Costa Mello et al., 2020, 2018b,a). Different crop species require nutrients in different quantities, for example the S requirement of members of the Brassicaceae family are higher than that of the Poaceae family. Therefore, some of the inconsistent results in the literature may be due to the range of different crops in which polyhalite has been investigated. However, even between similar tomato studies, changes in nutrient contents have been inconsistent (da Costa Mello et al., 2020, 2018b). The two tomato studies were grown in field settings, at a number of different locations and in different years. Many different variables can cause changes to soil nutrition including, rainfall, pH and the previous crop in the field and changes to such variables may have led to some the alterations reported in tomato nutrient content. Additionally, abiotic and biotic stresses to the plant may have caused alterations in nutrient content. The authors did identify that location had a significant effect on leaf and fruit nutrient concentrations (da Costa Mello et al., 2020). Therefore, further studies are required to help clarify results. It would also be beneficial if future studies included a wider range of nutrient measurements to help compare the results found in this study to other crop species.

In conclusion, fertiliser regimes containing polyhalite perform as well as KCl and K_2SO_4 fertiliser regimes in terms of plant growth in both balanced and unbalanced experiments. In general, the K fertiliser source had little or no impact on K nutrition whilst contents of a

number of macro and micronutrients varied inconsistently between experiments. Therefore, more studies both in the glasshouse and field as well as in a wider range of crop species are necessary to further elucidate these responses. Additionally, as rice cultivars can be adapted to different regions and soils, it may be possible that different cultivars respond differently to polyhalite as a K fertiliser and as such will be addressed in the next chapter.

Chapter 3: Investigating the effects of polyhalite on different rice cultivars

3.1 Introduction

Increasingly across the globe, K deficiency in soils is becoming a problem. It is reported that 3/4 of Chinese paddy fields and 2/3 of the Southern Australian wheat belt are deficient in K (Rengel & Damon, 2008). Continuation of agronomic practices that create nutrient imbalances can lead to soil nutrient mining and unbalanced fertiliser recommendations exacerbate these problems. Different rice cultivars are used across the globe, many of which are adapted to specific regions and climates. As well as displaying different grain characteristics, different cultivars also have a range of quality characteristics and possess different nutrient uptake and content characteristics. As such, it is clear that there are differences in cultivar ability to utilise different nutrients. Therefore, it is important to determine whether fertiliser composition also influences a cultivar's ability to take up different nutrients.

An example of the above can be found in differences that have been reported in the ability of *japonica* and *indica* subspecies of rice to utilise different N fertiliser forms. Both subspecies are able to access ammonium at a similar rate however *indica* species can accumulate more N when in it is provided as nitrate. The difference may be due to variation in the nitrate transporter gene *NRT1.1b (OsNPF6.5)* (Hu et al., 2015) allowing *indica* cultivars enhanced nitrate uptake. Given the differences seen in N uptake dependent on transporter properties, it is plausible that the diverse K uptake characteristics will respond differently to fertiliser composition, hence leading to rice cultivars responding differently to K sources.

Differences in cultivar ability to utilise different K fertiliser forms may also be influenced by the expression levels of different K transporters for example, AKT1 an inward-rectifying Shaker K^+ channel (Sentenac et al., 1992; Lagarde et al., 1996) or members of the KUP/HAK/KT and CHX gene families (Wang & Wu, 2010; White, 2013), which allow the uptake and internal transport of K.

Potassium use efficiency (KUE) refers to a cultivar's ability to utilise K compared to another cultivar and those classed as having a high KUE require less K for optimal growth and/or yield production. Generally, measurements of KUE fall into two categories, that of the ability of the plant roots to access and take up the K within the soil (KUpE) and the ability of the plant to convert the K it has procured into yield (KUtE) (White, 2013). It is believed that KUpE is mainly affected by a plant's capacity to produce a large root system, the ability of the roots to develop early and have high K uptake rates and the plant's production of exudates such as organic acids that can increase plant accessible K availability through the solubilisation of the pool of non-exchangeable K (Samal et al., 2010; White, 2013). A number of studies have shown the importance of root hairs in a plant's K uptake efficiency due to their ability to access a larger volume of soil (Høgh-Jensen & Pedersen, 2003; Samal et al., 2010). Furthermore in response to low K^+ conditions, crop plants modify the length of their root hairs allowing them to maximise uptake of K^+ from soluble sources by substantially increasing the root surface area (Høgh-Jensen & Pedersen, 2003).

Improvements in KUtE are mainly attributed to more efficient K transport around the plant and maintenance of optimal K concentrations in the cytosol. Some plants are also better at replacing K with other ions in non-essential roles which can help them survive periods of low K conditions and reallocating K from older leaves to young developing tissues. For example, other cations such as Na, Ca and Mg can all be used to balance vacuole charges, whilst sugars and organic acids can be used as a substitute osmoticum (White, 2013).

There are numerous different definitions for nutrient use efficiency due to the multitude of different nutrient sources available to plants, including from the soil, fertilisers and aerial deposition as well as the variety of factors which can influence crop nutrient demand including weather, genetics and crop management practices (Fixen et al., 2015). A summary of common calculations for nutrient use efficiency can be found in Table 3.1. One of the most frequently used calculations is: crop dry mass divided by plant potassium content (DMNP - Table 3.1). This definition has also been deemed one of the most reliable through the use of mathematical models (Moriconi & Santa-María, 2013).

Table 3.1 – Comparison of Nutrient Use Efficiency Calculations (adapted from Fixen et al 2015 with additional definitions noted with references in brackets)

Nutrient Use Efficiency Index	Calculation*	Question addressed
Dry Matter Nutrient Productivity (DMNP) (Moriconi & Santa-María, 2013)	DMNP = DM / U	How is the crop biomass production compared to the plants' nutrient content?
Dry Matter Utilisation Efficiency (DMUE) (White et al., 2013)	DMUE = DM / F	What is the plants' ability to transform fertiliser input into biomass?
Internal Utilisation Efficiency (IE)	IE = Y/U	What is the plants' ability to transform nutrients acquired from all sources into economic yield?
Physiological Efficiency (PE)	PE = $(Y - Y_0) / (U - U_0)$	What is the plants' ability to transform nutrients from the fertiliser applied into economic yield?
Fertiliser Use Efficiency (FUE)	FUE = U/F	What if the efficiency of nutrient capture?
Agronomic Efficiency (AE)	AE = $(Y - Y_0) / F$	How much did yield increase in comparison to the fertiliser input?
Partial Factor Productivity (PFB)	PFP = Y/F	How productive is the cropping system compared to the fertiliser input?
Partial Nutrient Balance (PNB)	PNB = U_H / F	How much nutrient is being removed from the system compared to how much is applied?
Apparent Recovery Efficiency (RE)	RE = $(U - U_0) / F$	How much of the applied nutrient did the plant take up?
Shoot Nutrient Productivity (SUE) (Hartley et al., 2020)	SUE = RGR / U_S	How is the crop shoot biomass production compared to the plants' nutrient content?

*DM = plant dry matter, Y = yield, Y_0 = yield without nutrient, F = fertilizer applied, U_H = Nutrient in harvested parts, U = nutrients in above ground biomass with nutrient applied, U_0 = nutrient in above ground biomass with no nutrient applied, U_S – nutrient in shoot, RGR = relative growth rate

Potassium fertiliser composition may also impact on plant KUE, as demonstrated in cotton, where slow release K fertilisers improved plant production and KUE (Tian et al., 2017) as well as increasing yield (Chen et al., 2021). The genetic differences in KUE between species and accessions have been recognised for some time (White, 2013; Wang & Wu, 2015), however it is only recently that studies have begun identifying the responsible genes. Variation in KUE has been seen in *Brassica oleracea* with White et al. (2010) observing more than 2-fold variation in the levels of shoot K between accessions, as well as in *Arabidopsis thaliana* (Chao et al., 2013) and rice cultivars. In a study of 134 rice cultivars, Yang et al. (2003) found substantial genotypic differences in plant KUtE with close to 6-fold variation between cultivars when investigating DMUE and although these increases were smaller between genotypes when investigating IE. Further investigations into rice KUtE calculated using SUE and a genome wide association study (GWAS) identified the high-affinity K transporter, *Os*HKT2:1 gene as having a distinguishing role in cultivar KUE. The *Os*HKT2:1 transport which physiologically is thought to actually function as an Na transporter, was more highly expressed in high KUE cultivars compared to those which were identified as having a low KUE (Hartley et al., 2020).

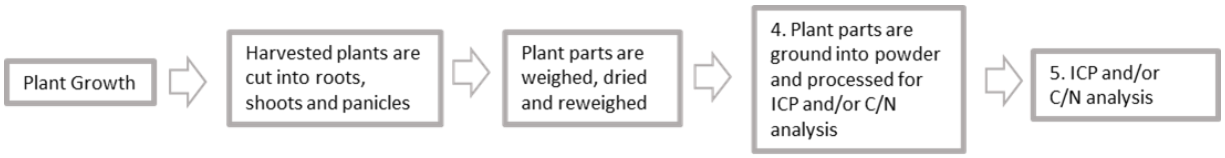
In this study, a range of rice cultivars (Table 3.2), previously identified as having high or low KUE (Hartley, 2018; Hartley et al., 2020) was used to determine whether rice growth traits, yield, or nutrient content varied in response to different K sources. The results of this experiment were evaluated to determine firstly how a range of rice cultivars responded to different K sources and secondly were grouped into low and high KUE categories to determine how collectively cultivars with a different KUE responded to different K sources.

Another important factor in K fertilisation is the content of chloride ions in the world's most popular K source, KCl. As many crop species are sensitive to chloride ions it is necessary to determine whether rice cultivars respond negatively to Cl ions at low concentrations and therefore whether polyhalite could act as a more beneficial K fertiliser for rice growth. The rice cultivars described above were also used to determine whether different levels of Cl ions affected the growth of rice plants.

Table 3.2 – KUE of Cultivars used. Low KUE lines are in bold

Cultivar (GSOR ID)	SUE (relative growth rate / shoot K content) (from Hartley et al., 2020)
042	**0.031**
115	**0.030**
133	0.074
357	0.074
377	**0.033**
401	**0.029**

3.**2 Methods**



3.2.1 Growth Methods

3.2.1.1 Investigating the use of polyhalite as a potassium fertiliser in pots and determining cultivar differences in growth

Rice seeds of the cultivars; Dom Sufid (L) (GSOR 301-042), Pagaiyahan (GSOR 301-115) (L), Lady Wright Seln (L) (GSOR 301-377), IR64 (L) (GSOR 301-401), Shai-Kuh (GSOR 301-133) (H) and Coarse (H) (GSOR 301-357), were sown and germinated in sand with deionised water. Cultivars are subsequently referred to by the number after the "GSOR 301- "for convenience e.g. "GSOR 301-042" → "042". Low KUE cultivars are marked with an (L) whilst high KUE are marked with an (H). After two weeks, 24 seedlings of each cultivar were transferred to 9 cm x 9 cm x 9 cm pots filled with 1.4 kg sand, with 2 plants per pot. Pots were placed in circular, plastic, lidded containers which were 4 cm high with a 14 cm diameter and were covered in black tape to reduce evaporation. Plants were fed on the day of transplanting and then twice more at 3 weeks and 6 weeks after transfer to pots. Fertiliser treatments were an adjusted yoshida (AY2) and a polyhalite solution made from polyhalite powder (Poly) (AY2 and PP in table 2.3). Treatments were arranged in a complete randomised block. Eight plants were destructively harvested at 7 days after final fertiliser addition (vegetative), after the first

panicle fully emerged (flowering) and one month after the first panicle had fully emerged (grain). This complete experimental design was repeated 3 times at different timepoints in the year to reduce seasonal effects (n = 72 per cultivar with 24 of each cultivar per harvesting timepoint).

3.2.1.2 Investigating the impact of chloride ions on rice growth and nutrient content in different rice cultivars

Rice seeds of the cultivars; Dom Sufid (042), Pagaiyahan (115), Shai-Kuh (133) and Coarse (357) were sown and germinated in sand with deionised water. After two weeks 12 plants of each cultivar were transferred into four 1.2 L hydroponics boxes with 3 plants of each cultivar per box and each box containing 12 plants. Cultivars were organised in a complete randomised block within the box. Each box contained a different nutrient solution based on yoshida with chloride concentrations of 0 mM, 2 mM, 3 mM and 6 mM in the different treatments with corresponding changes in the Ca content of 1 mM, 1 mM, 1 mM, and 3 mM (table 3.3) (Yoshida et al., 1976). Nutrient solutions were replaced weekly. This complete experimental design was repeated 3 times at different timepoints in the year to reduce seasonal effects (n = 36 per cultivar and 9 of each cultivar per treatment).

Table 3.3 – Nutrient concentrations for experiment 3.2.1.2

Nutrient	Chemical providing nutrient	Concentration of nutrient in 0mM chloride solution (mM/l)	Concentration of nutrient in 2mM chloride solution (mM/l)	Concentration of nutrient in 3mM chloride solution (mM/l)	Concentration of nutrient in 6mM chloride solution (mM/l)
N	NH_4NO_3	2.9	2.9	2.9	2.9
P	$NaH_2PO_4.2H_2O$	0.3	0.3	0.3	0.3
K	K_2SO_4 KCl	1 -	1 -	0 2	1 -
Ca	$CaCl_2$ $CaSO_4.2H_2O$	- 1	1 -	1 1	3 -
Mg	$MgSO_4.7H_2O$	1.6	1.6	1.6	1.6
S	K_2SO_4 $MgSO_4.7H_2O$ $CaSO_4.2H_2O$	1 1.6 1	1.6 1 -	- 1.6 0.5	1 1.6 -
Cl	KCl $CaCl_2$	- -	- 2	2 1	- 6

3.2.2 General Methods

3.2.2.1 Glasshouse conditions

Plants were grown in a glasshouse in 12 hour day/night cycle. Day temperatures were 28-32°C and night temperatures were 24-30°C.

3.2.2.2 Growth analyses

All seedlings were weighed before transferral to pots. Harvested plants were split into roots and shoots, weighed and dried in a fan oven at 80°C for 72 hours, after which they were reweighed.

3.2.2.3 Measurement of nutrient content for 3.2.1

Dried root, shoot and panicle samples from plants sampled at senescence were ground into a powder using Ø 5 mm carbon steel balls and a Ball mill (Retsch mm300). The two plants from each pot were pooled with 20 mg of ground sample used from each plant. Where only one plant had survived in a pot, only this plant was sampled and future measurements were adjusted accordingly. Samples were digested with HNO_3 for 24 hours at 70 °C. Digested samples were then diluted with ultra-pure water and filtered. Samples were then run through a Thermo iCAP 7400 Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES) (Thermo Fisher) to determine concentrations of K, Ca, Mg, S, P, Na, Fe, Mn, Zn, B and Cu. In addition to individual nutrient analyses, total macronutrient and micronutrient calculations were made by adding together the concentrations of the macronutrients consisting of: K, Mg, S, Ca, P and Na or micronutrients: Fe, B, Cu, Mn and Zn.

3.2.2.4 Statistical analysis

Visual inspection of the data led to outliers being removed before statistical analysis. All statistical analyses were performed using R software (version 3.6.1, R Core Team, 2019). Graphs were produced using the *ggplot2* package (Wickham, 2016). Significance was set at $p < 0.05$ for all weight and nutrient data.

ANOVA tests were used to test the main and interactive effects between the treatments and cultivars, followed by a Tukey post hoc test to determine significant interactions when they were apparent. Replicate was included in ANOVA tests as an independent variable. When data did not fit the assumptions of an ANOVA, attempts were

made to normalise the data through log, logit and square rooting or cube rooting the data. When results did not fit the assumptions of an ANOVA, Kruskal-Wallis tests were used to test the main effects between AY and polyhalite treatments and the main effects between cultivars, followed by a Dunn's post hoc test to determine significant interactions when they were apparent.

Type 3 ANOVA tests were used to test the main and interactive effects between the treatments and cultivars grouped by KUE, followed by a Tukey post hoc test to determine significant interactions when they were apparent. Replicate was included in ANOVA tests as an independent variable. Using a type 3 ANOVA weighted the test to help remove the imbalance in the number of samples between the groupings. When data did not fit the assumptions of an ANOVA, attempts were made to normalise the data through log, logit, square rooting or cube rooting the data. When results did not fit the assumptions of an ANOVA, Kruskal-Wallis tests were used to test the main effects between AY and polyhalite treatments and the main effects between cultivars, followed by a Dunn's post hoc test to determine significant interactions when they were apparent (see supplementary information for further details of statistical tests used).

3.3 Results

3.3.1 Investigating the use of polyhalite as a potassium fertiliser in sand with a range of rice cultivars

3.3.1.1 Growth analyses

A range of different rice cultivars was grown in pots containing sand to determine whether plant growth responded to a fully balanced nutrient solution (AY2) containing chemical salts matching the polyhalite nutritional profile compared to a polyhalite powder solution (Poly). Each pot was provided with the same concentration of each nutrient. Rice cultivars were chosen on the basis of varying KUE (table 3.2) according to the study by Hartley et al., 2020. Growing plants in sand allowed for easier measurement of root traits as well as removing any additional complexity and nutrients that a soil system would supply. No significant differences were seen in dry weights of roots (Kruskal Wallis, $p < 0.05$), shoots (vegetative stage – two-way ANOVA, flowering and grain stages - Kruskal Wallis, $p < 0.05$) or

panicles (flowering stage - cube root transformed two-way ANOVA, grain stage -Kruskal Wallis, $p < 0.05$) between the treatments at any of the three harvesting stages (fig. 3.1).

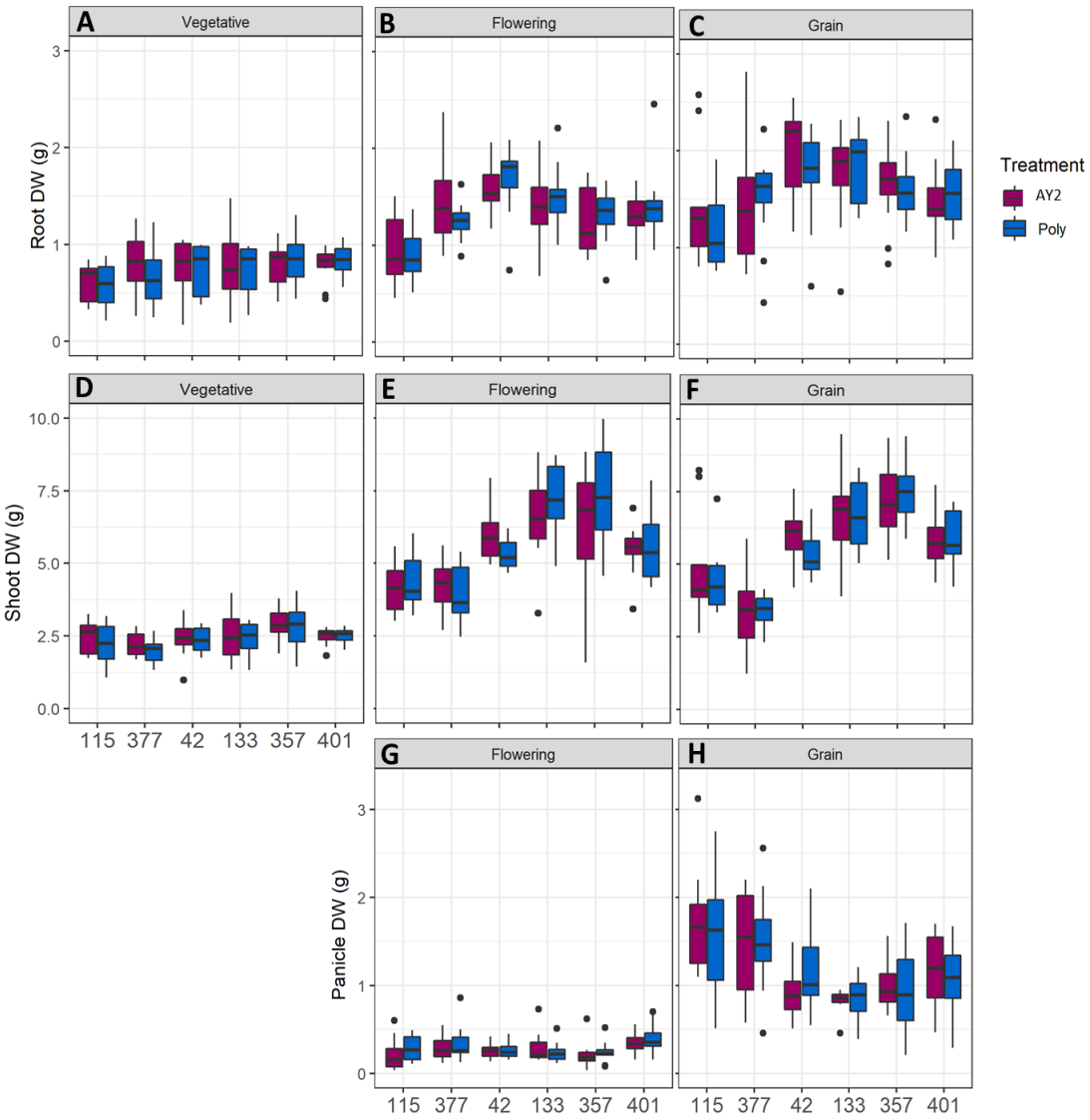


Figure 3.1 – Dry weights of roots, shoots and panicles of different rice cultivars - Boxplots of dry weights of root (A, B, C), shoot (D, E, F) and panicle (G, H) of plants grown in pots with an adjusted yoshida (AY2) or a polyhalite powder solution (Poly). Plants were sampled at three different lifecycle stages (see fig.2.1. caption for explanation of boxplot). No significant differences ($p < 0.05$, ANOVA or Kruskal-Wallis) were seen between the treatments in any plant part or at any sampling stage.

3.3.1.2 Nutrient analyses

Cultivars were also tested to determine if their nutrient composition was affected by treatment. Whilst the averages of the combined cultivars had significant differences in the content of some nutrients, no significant interactions were found between the treatment and cultivars. At the vegetative stage, plants grown in the polyhalite treatment had a significantly higher Fe (two-way ANOVA, $p = 0.002$) and Na (Kruskal Wallis, $p = 0.018$) content in their shoots (figs. 3.2 and 3.8), however the shoot K (Kruskal Wallis, $p = 0.0094$) and B (Kruskal Wallis, $p = 0.03$) content was significantly lower in the polyhalite treatment than the AY2 treatment (figs. 3.2, 3.6 and 3.8). Lower root Cu (log transformed two-way ANOVA, $p = 0.024$) was also observed in the polyhalite treatment compared to the AY2 treatment (figs. 3.5 and 3.8). All other nutrients were not significantly different between treatments at this sampling stage (appendix figures 6.2.1 – 6.2.11).

At the flowering stage, plants grown in the polyhalite treatment had a significantly lower K shoot content (two-way ANOVA, $p = 0.00039$) and lower Fe panicle content (figs. 3.3, 3.4 and 3.9) than the AY2 treatment (Kruskal Wallis, $p = 0.036$). The difference in K content of the shoot at this sampling stage was also highly significant between cultivars (two-way ANOVA, $p = 1.98 \times 10^{-4}$), however the interactive effect between treatment and cultivar was not significant. Additionally, the total macronutrient content per dry weight was significantly lower in the shoots of plants grown with the polyhalite treatment compared to the AY2 treatment (two-way ANOVA, $p = 0.019$) as was the total micronutrient content per dry weight in the panicles of plants (log transformed two-way, ANOVA, $p = 0.01$).

At the grain stage, plants grown with the polyhalite treatment had a significantly higher panicle Ca content (Kruskal Wallis, $p = 0.04$), however the shoots of these plants had a significantly lower Mg content (Kruskal Wallis, $p = 0.016$) compared to the AY2 treated plants (3.3, 3.4 and 3.9). The Mg content of the shoot at this sampling stage also had significant differences between cultivars (Kruskal Wallis, $p = 0.003$) however the interactive effect between cultivar and treatment was not significant. Additionally, the total macronutrient content per dry weight was significantly lower in the shoots of plants grown with the polyhalite treatment (two-way ANOVA, $p = 0.017$) as well as there being a significant

difference between the interaction of treatment and cultivar ($p = 0.0065$). The overall total of nutrients per dry weight measured at the grain stage was also significantly lower in the shoots of plants grown with the polyhalite treatment (two-way ANOVA, $p = 0.018$) as well as there being a significant difference in the interaction of treatment and cultivar ($p = 0.007$). The interaction seen between treatment and cultivar in both the total macronutrient and overall nutrient content per dry weight was explained by differences in cultivar response to the different treatments. No significant differences were observed within an individual cultivar's responses to the different treatments.

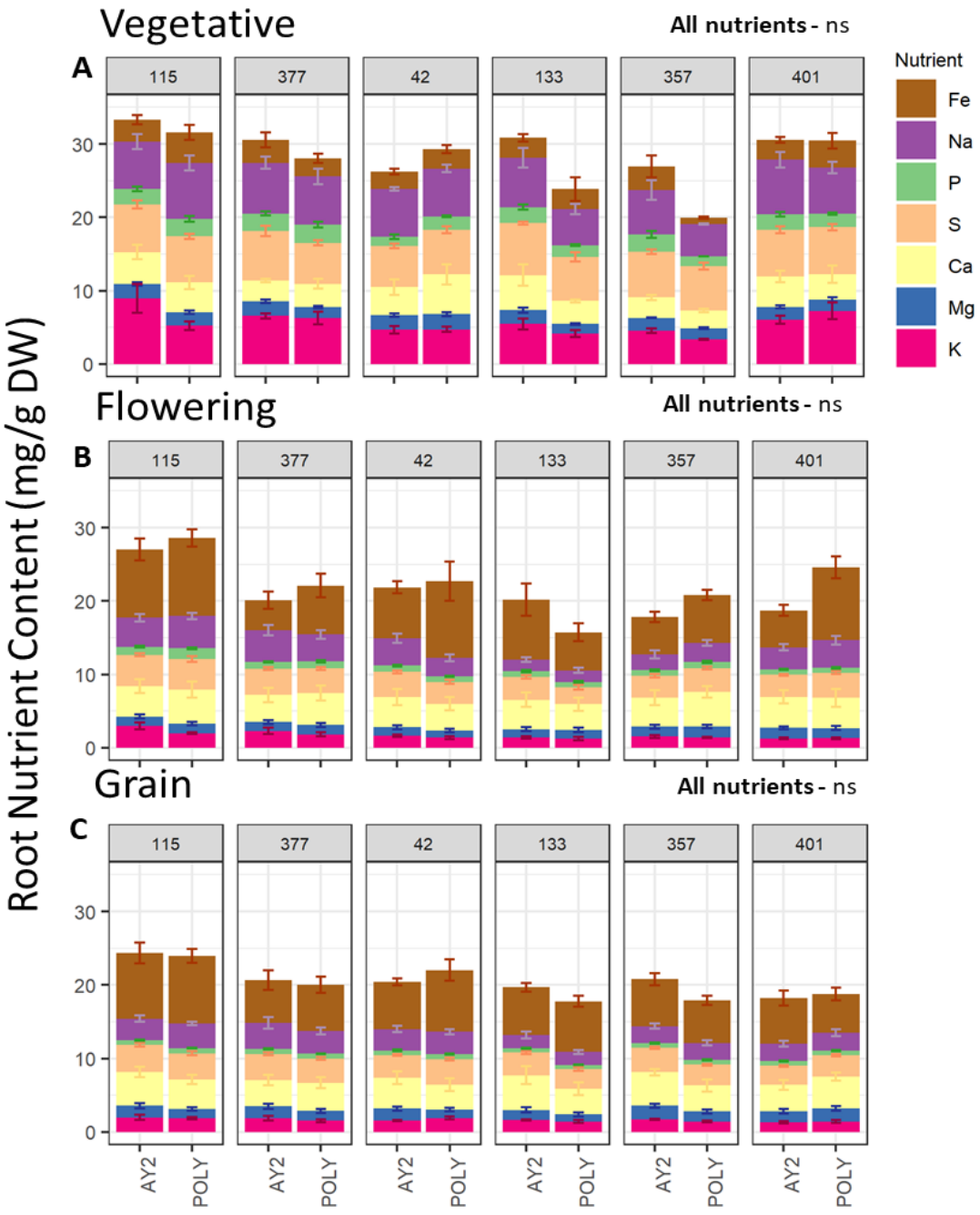


Figure 3.2 – Root macronutrient and iron content of six rice cultivars. Stacked barplots of nutrient content of roots at three sampling stages, vegetative (A), flowering (B) and grain (C). Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. No significant differences (ANOVA or Kruskal Wallis, $p < 0.05$) were seen in nutrient contents in any cultivar as noted in the top right corner above each panel of cultivar plots. Each nutrient barplot represents the mean of twelve plants with error bars denoting the standard error.

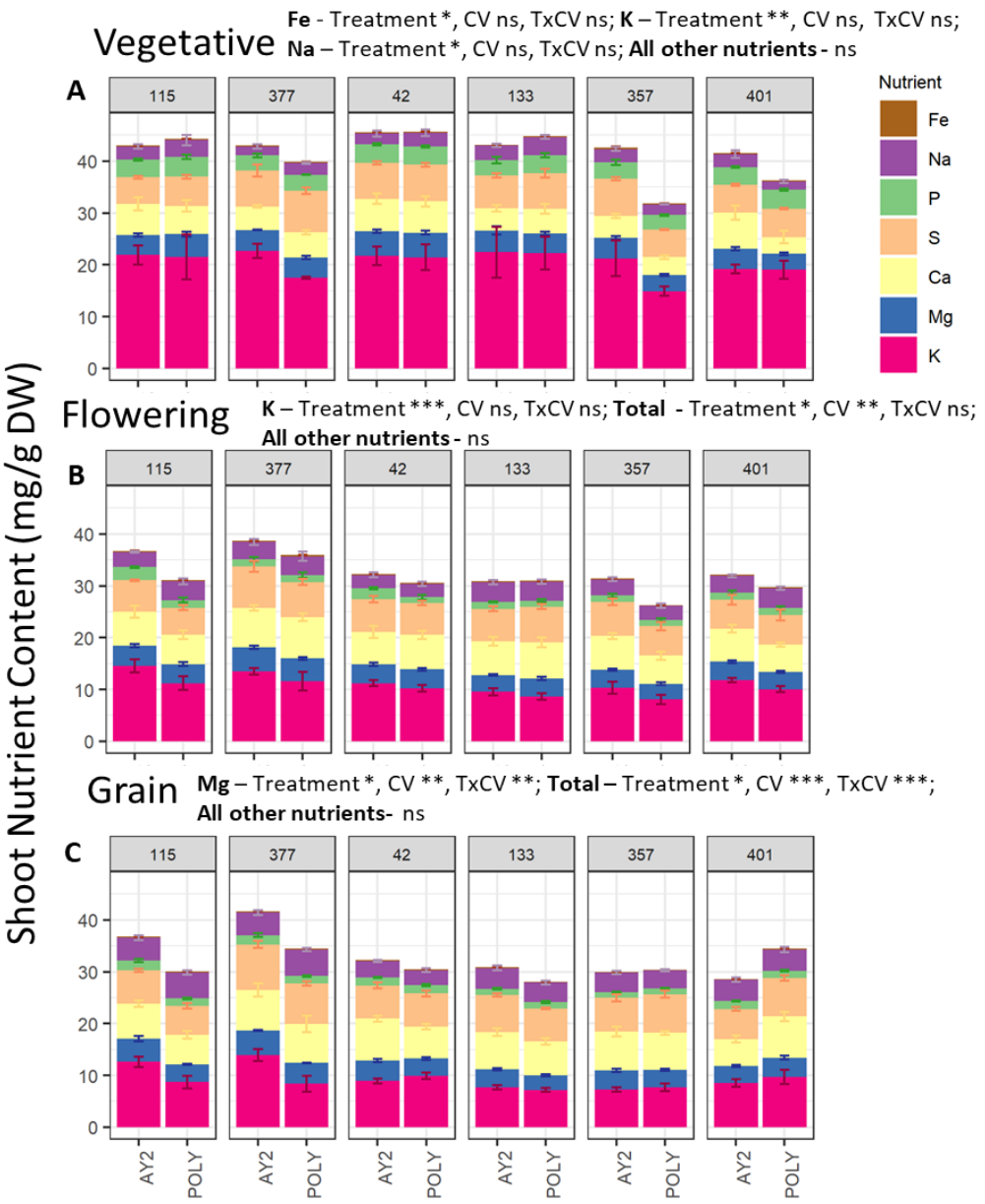


Figure 3.3 – Shoot macronutrient and iron content of six rice cultivars. Stacked barplots of nutrient content of shoots at three sampling stages, vegetative (A), flowering (B) and grain (C). Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) seen in the nutrient contents between treatments, cultivar (CV) and interactive effects (TxCV) are noted by asterisks in the top right corner above each panel of cultivar plots ((* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$). Each nutrient barplot represents the mean of twelve plants with error bars denoting the standard error.

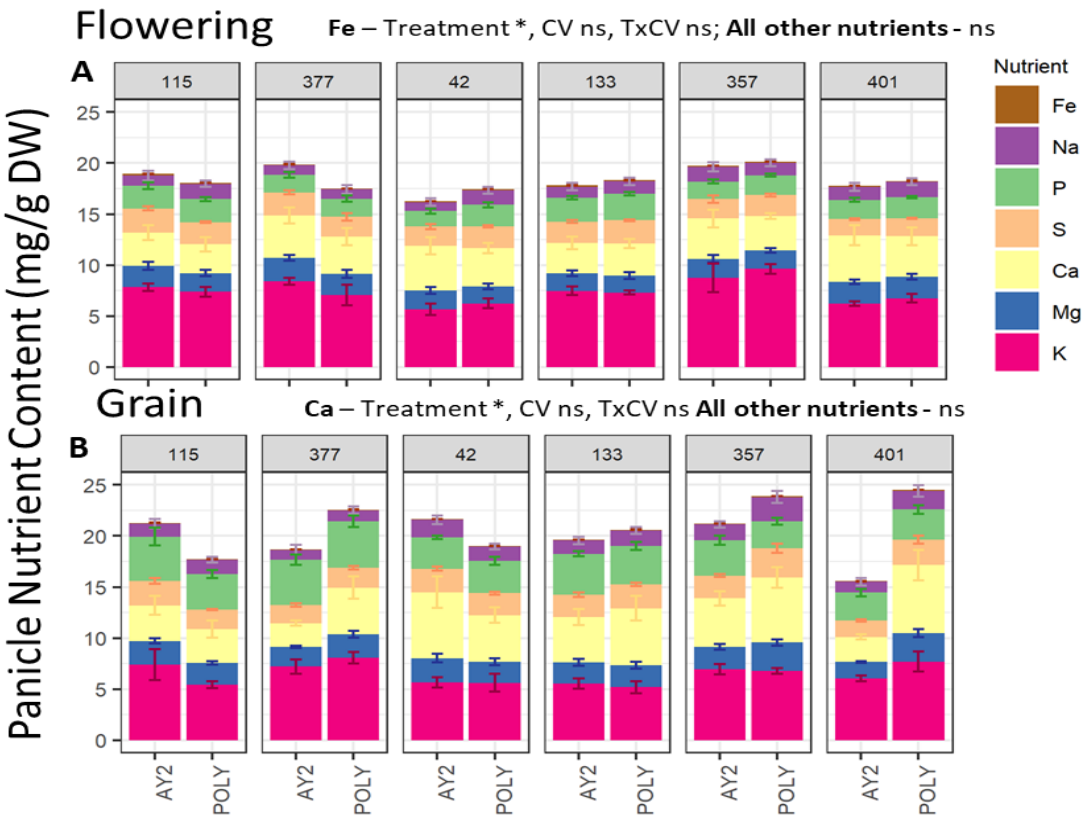


Figure 3.4 - Panicle macronutrient and iron content of six rice cultivars. Stacked barplots of nutrient content of panicles at two sampling stages, flowering (A) and grain (B). Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) seen in the nutrient contents between treatments, cultivar (CV) and interactive effects (TxCV) are noted by asterisks in the top right corner above each panel of cultivar plots (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$). Each nutrient barplot represents the mean of twelve plants with error bars denoting the standard error.

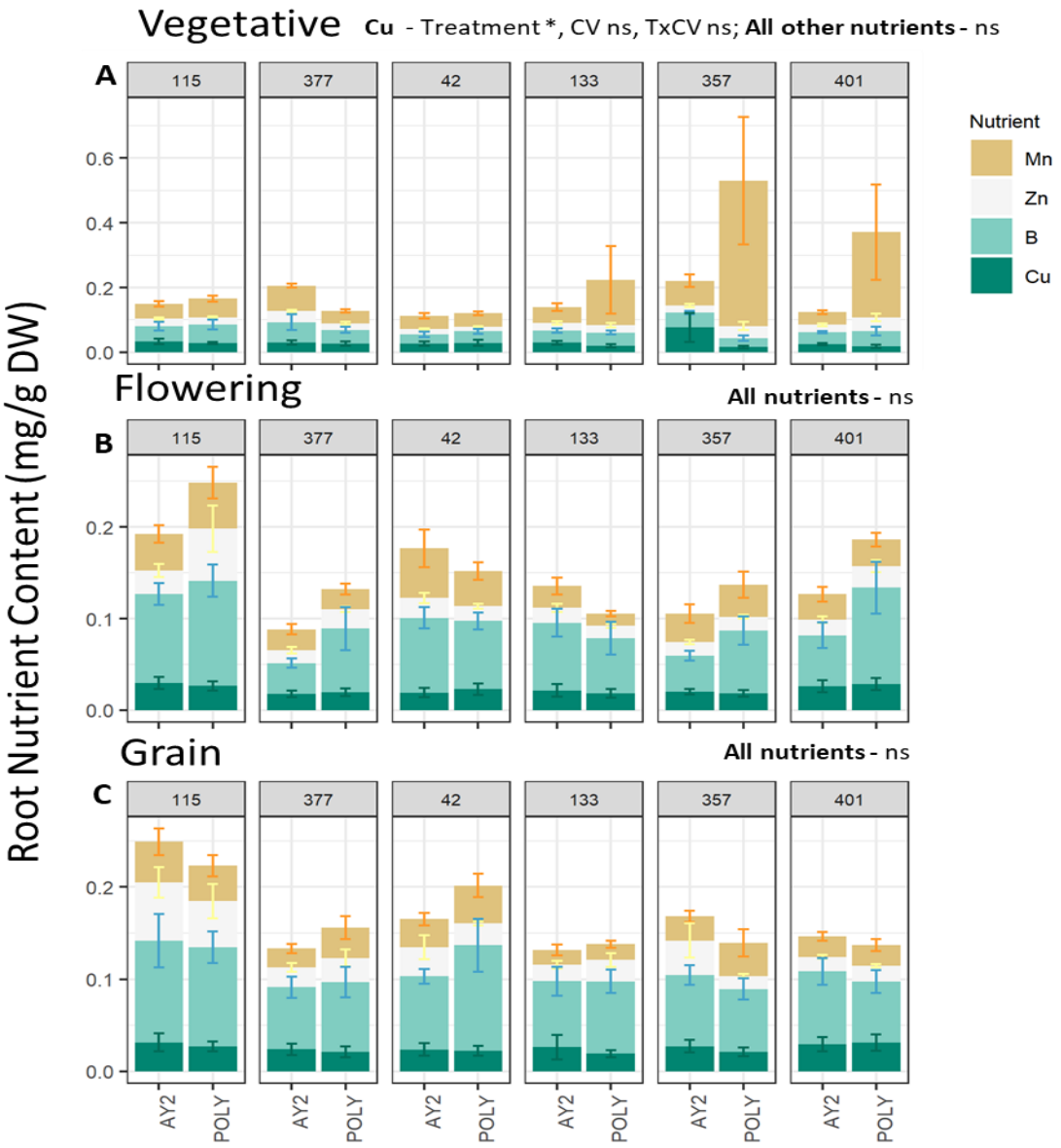


Figure 3.5 – Root micronutrient content of six rice cultivars. Stacked barplots of nutrient content of roots at three sampling stages, vegetative (A), flowering (B) and grain (C). Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. No significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) were seen in the nutrient contents between treatments as noted in the top right corner above each panel of cultivar plots. Each nutrient barplot represents the mean of twelve plants with error bars denoting the standard error.

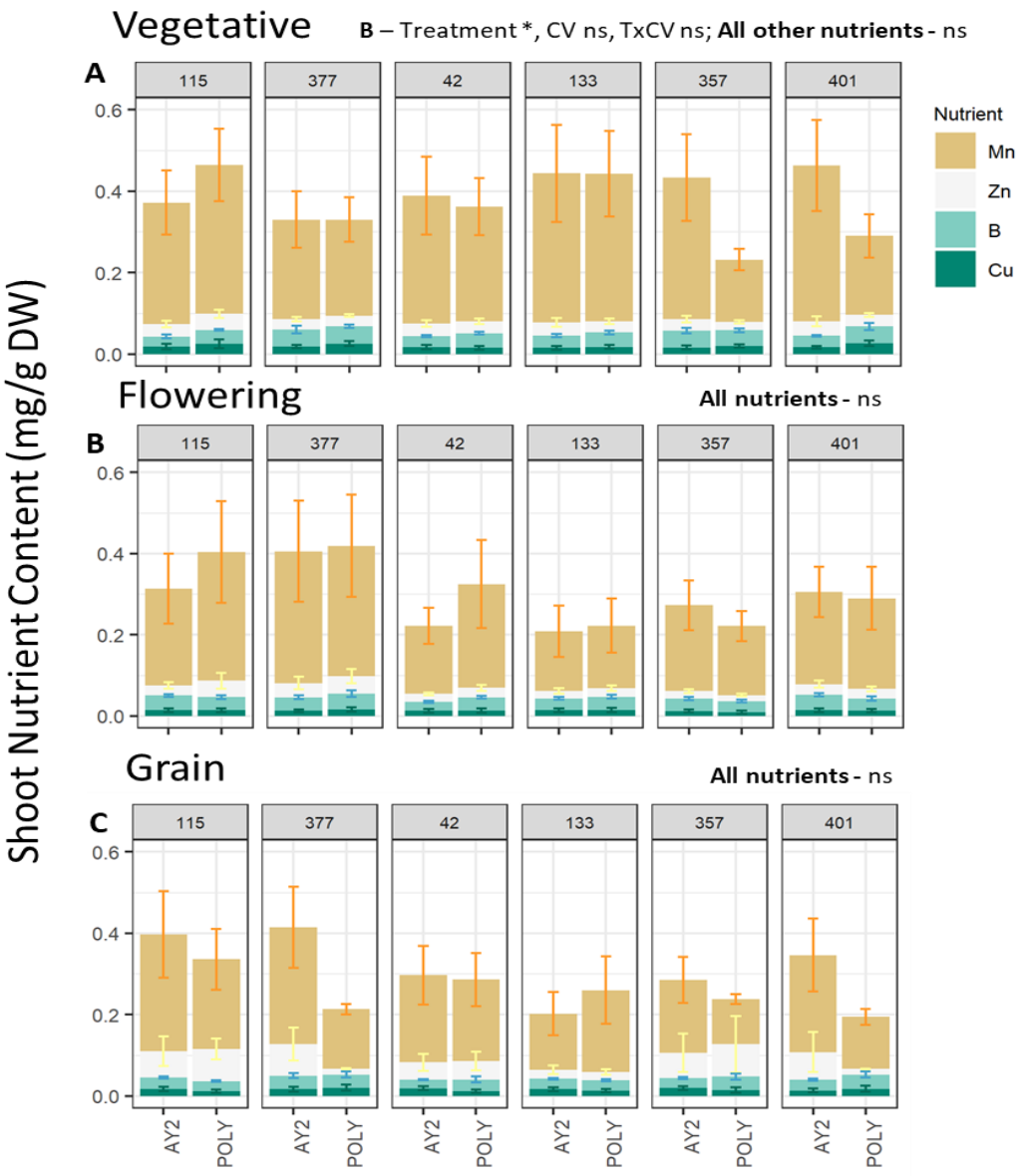


Figure 3.6 – Shoot micronutrient content of six rice cultivars. Stacked barplots of nutrient content of shoots at three sampling stages, vegetative (A), flowering (B) and grain (C). Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) seen in the nutrient contents between treatments, cultivar (CV) and interactive effects (TxCV) are noted by asterisks in the top right corner above each panel of cultivar plots ((* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$). Each nutrient barplot represents the mean of twelve plants with error bars denoting the standard error.

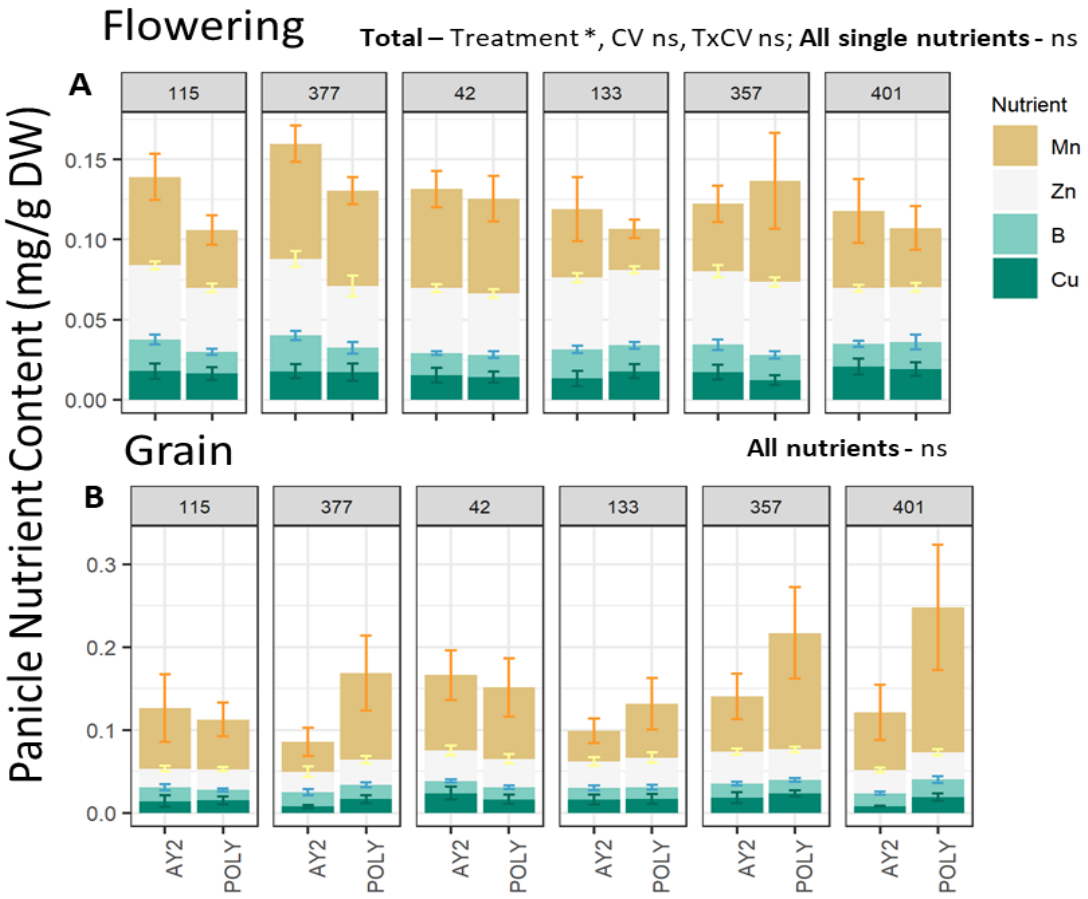


Figure 3.7 – Panicle micronutrient content of six rice cultivars. Stacked barplots of nutrient content of panicles at two sampling stages, vegetative (A), flowering (B) and grain (C). Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) seen in the nutrient contents between treatments, cultivar (CV) and interactive effects (TxCV) are noted by asterisks in the top right corner above each panel of cultivar plots ((* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$). Each nutrient barplot represents the mean of twelve plants with error bars denoting the standard error.

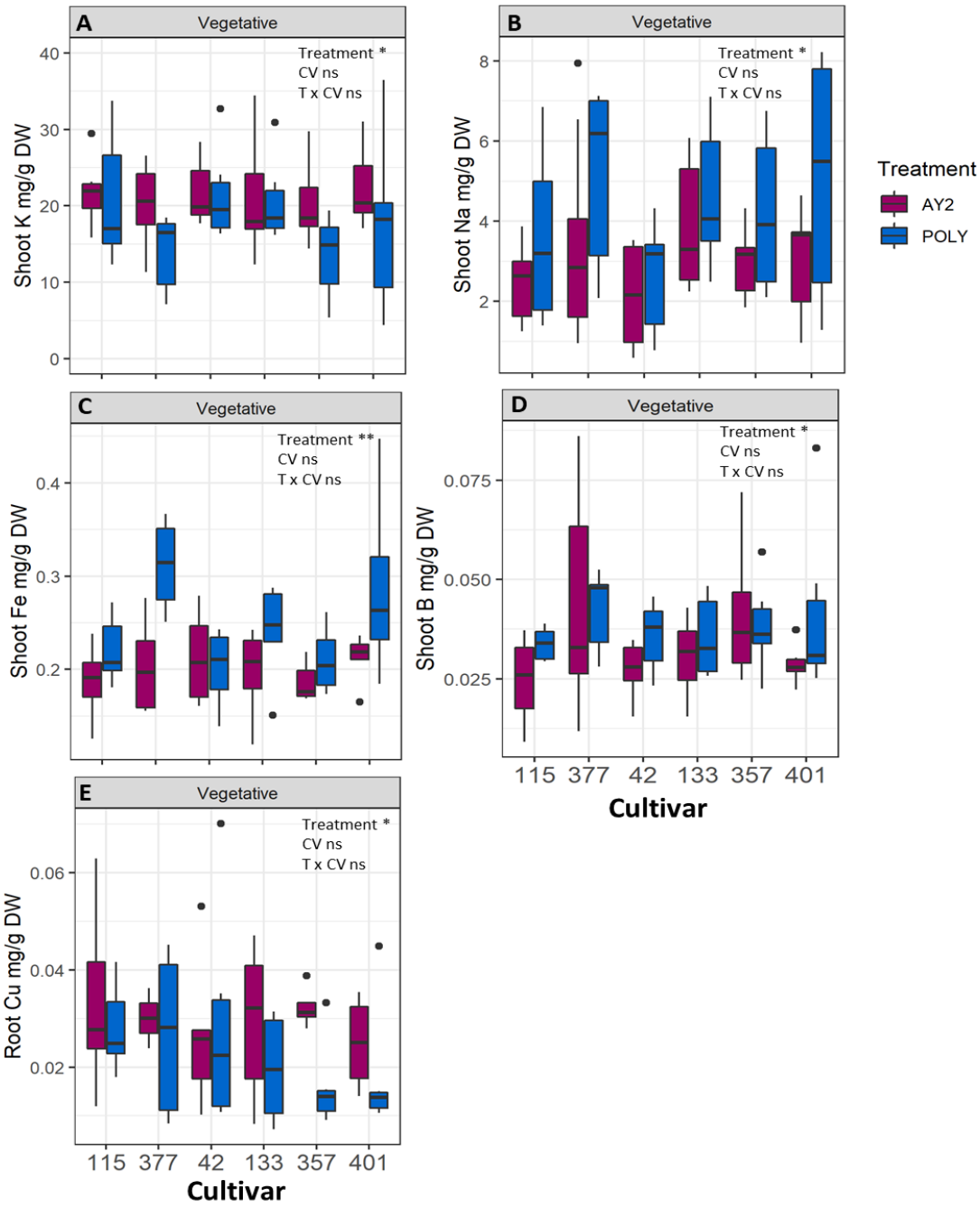


Figure 3.8 – Individual nutrient contents at the vegetative stage with significant differences of six rice cultivars. Boxplots of shoot K (A), Na (B), Fe (C), B (D) and root Cu (E). These nutrients had significantly different nutrient content between treatments, cultivar (CV) or interaction between treatment and cultivar (T x CV) of plants grown with an adjusted yoshida (AY2) and polyhalite powder (Poly) treatment. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar or interaction between treatment and CV are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

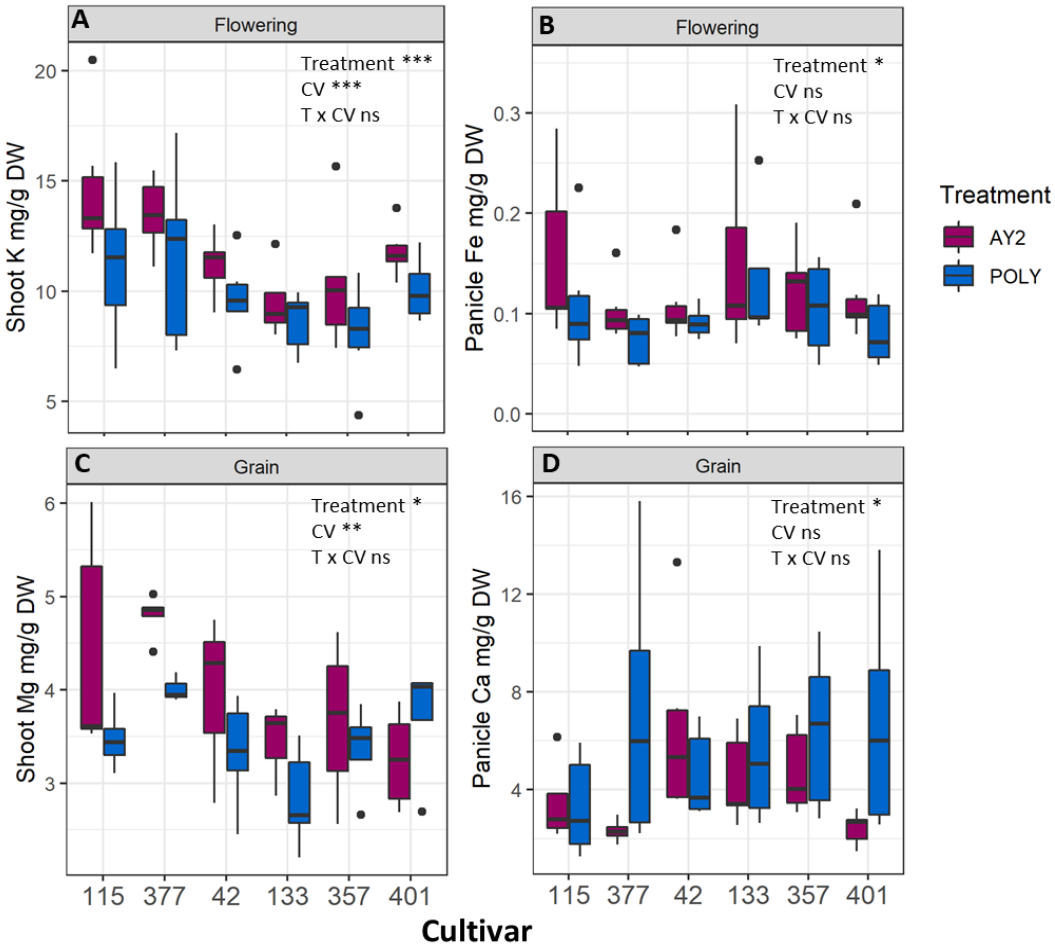


Figure 3.9 – Individual nutrient contents at the flowering and grain stages with significant differences of six rice cultivars. Boxplots of shoot K (A) and panicle Fe (B) at the flowering stage and shoot Mg (C) and panicle Ca (D) at the grain stage. These nutrients had significantly different nutrient content between treatments, cultivar (CV) or interaction between treatment and cultivar (T x CV) of plants grown with an adjusted yoshida (AY2) and polyhalite powder (Poly) treatment. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar or interaction between treatment and CV are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

3.3.2 Investigating the use of polyhalite as a potassium fertiliser in sand with a range of rice cultivars grouped by KUE

3.3.2.1 Growth analyses

The cultivars described above were grouped into a low and high KUE category (table 3.4) with 115 and 377 in the low category and 042, 133, 357 and 401 in the high to determine if this categorisation had any effect on plant growth traits. The calculation used to determine KUE was total crop dry weight divided by total K content. This calculation grouped the low and high KUE cultivars differently from the groupings given by Hartley et al 2020 as indicated in bold in table 3.3.

No significant differences were seen in the average dry weights of roots (vegetative stage – Kruskal Wallis, flowering and grain stage – type 3 two-way ANOVA, $p < 0.05$), shoots (vegetative and flowering stage – Kruskal Wallis, grain stage – type 3 two-way ANOVA, $p < 0.05$) or panicles (flowering stage – cube root transformed – type 3 two-way ANOVA, grain stage – Kruskal Wallis, $p < 0.05$) between the treatments at any of the three harvesting stages (fig. 3.10). However, the cultivars in the low KUE category had significantly lower root dry weights than the high KUE cultivars at all sampling stages (vegetative stage – Kruskal Wallis $p = 0.0058$, flowering and grain stage – type 3 two-way ANOVA, $p = 0.019$ and 0.011) and lower shoot dry weights at the flowering and grain sampling stages (flowering stage – Kruskal Wallis $p = 5.36 \times 10^{-7}$, grain stage – type 3 two-way ANOVA, $p = 8.95 \times 10^{-15}$). Conversely, the low KUE cultivars had higher panicle dry weights than the high KUE cultivars at the grain sampling stage (Kruskal Wallis, $p = 4.96 \times 10^{-8}$). At the grain stage the interaction between KUE and treatment in the shoot was significant (Kruskal Wallis, $p = 4.512 \times 10^{-13}$) with differences in the low and high KUE cultivar responses to the AY2 treatment (Dunn's post-hoc test, $p = 3.6 \times 10^{-6}$) and polyhalite treatment (Dunn's post-hoc test, $p = 1.94 \times 10^{-7}$). In both cases the high KUE cultivars had higher shoot dry weights with both treatments than the low KUE cultivars. A significant interaction between KUE and treatment was also seen at the grain stage in the panicle dry weights (Kruskal Wallis, $p = 1.26 \times 10^{-6}$) again with differences in the low and high KUE cultivar responses to the AY2 treatment (Dunn's post-hoc test, $p = 2.7 \times 10^{-4}$) and polyhalite treatment (Dunn's post-hoc test, $p = 0.0017$). These interactive effects reflect the that in both treatments the low KUE cultivars had higher panicle dry weights than the cultivars

in the high KUE category. No significant differences between treatments were seen within the low or high KUE categories.

Table 3.4 Average KUE for different cultivars. The average KUE of rice cultivars from experiment 3.2.1.1 compared to KUE values from previous research. The numbers in bold indicate the low KUE lines for each KUE calculation.

Cultivar	KUE (total crop dry weight/ total K content)	KUE (relative growth rate / shoot K content) (from Hartley et al., 2020)
042	0.357	**0.031**
115	**0.262**	**0.030**
133	0.42	0.074
357	0.429	0.074
377	**0.254**	**0.033**
401	0.339	**0.029**

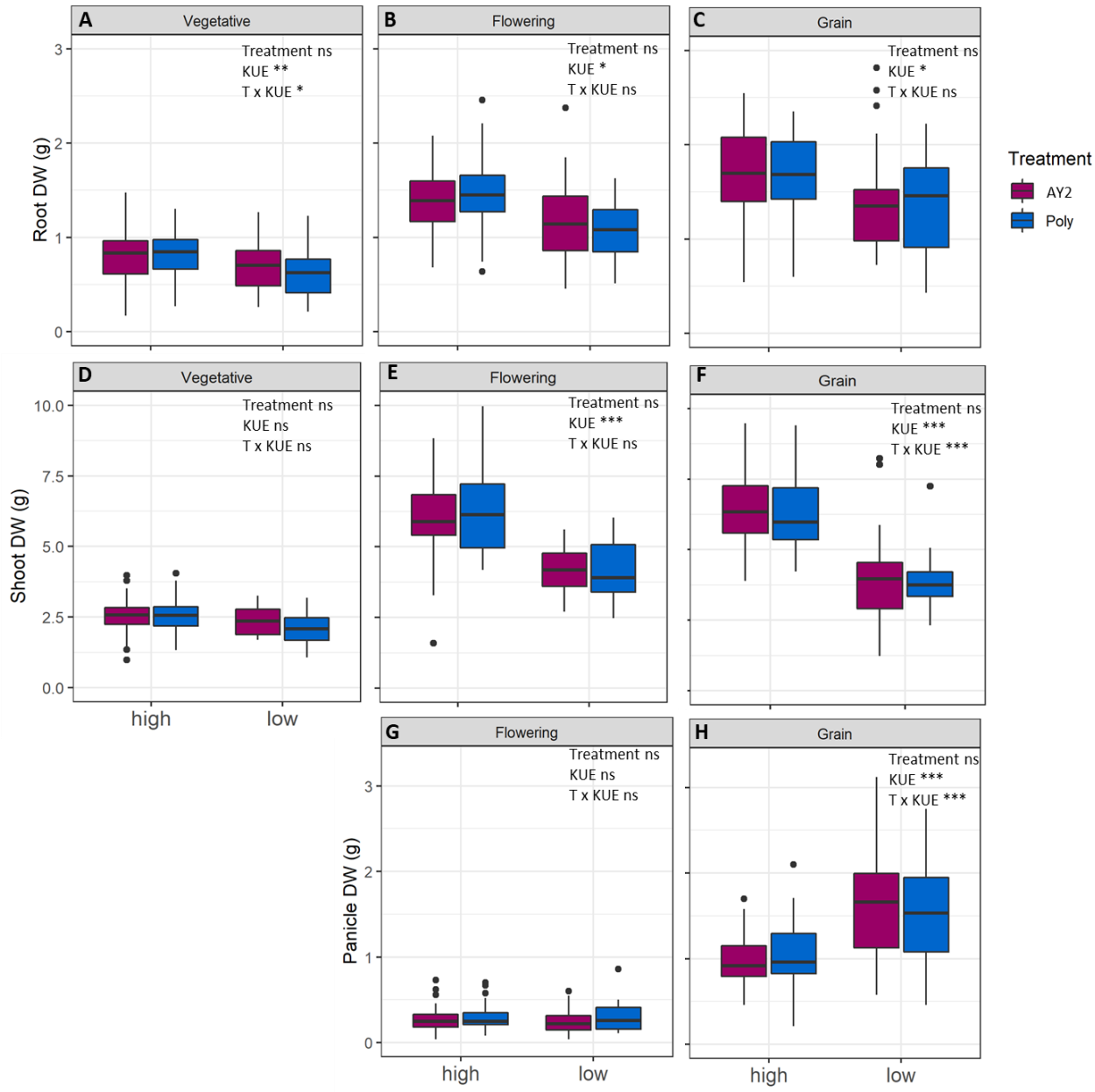


Figure 3.10 – Dry weights of roots, shoots and panicles of different rice cultivars grouped by KUE. Boxplots of dry weights of root (A, B, C), shoot (D, E, F) and panicle (G, H) of plants grown in pots with an adjusted yoshida (AY2) and polyhalite powder solution (Poly). Plants were sampled at three different lifecycle stages and grouped by high and low KUE type (see fig.1. caption for explanation of boxplot). Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) seen in the nutrient contents between treatments, KUE and interactive effects (TxKUE) are noted by asterisks in the top right corner above each panel of cultivar plots ((* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$).

3.3.2.1 Nutrient analyses

Nutrient composition of cultivars was grouped by KUE and analysed to determine the effect of treatment on these measurements. At all sampling stages the nutrient content results when cultivars were grouped by KUE (figs. 3.11 – 3.15) were mostly consistent with those obtained for individual cultivars (figs. 3.2 – 3.9). Grouping by KUE removed the significant difference seen between treatments in the Mg shoot content at the grain sampling stage (type 3 two-way ANOVA, $p < 0.05$), whereas lower root Na content was seen at the vegetative stage in the polyhalite treated plants than the AY2 treated plants (type 3 two-way ANOVA, $p = 0.007$). Additionally, more interactions between treatment and KUE were observed when cultivars were grouped (tables 3.5-3.12). At the vegetative stage, significant interactions were found in the content of root K (Kruskal Wallis, $p = 0.032$) as well as shoot K content (Kruskal Wallis, $p = 0.036$), however in both of these cases the interaction was not of interest to this study with neither the different KUE groupings having significantly different responses to each other or to the treatment nor the individual KUE groupings having significantly different responses to the treatments. Significant differences in the interactive effect were also seen in root Na content (type 3 two-way ANOVA, $p = 0.033$) at the vegetative stage. Here the low and high KUE groupings had significantly different responses to the polyhalite treatment (Tukey's range test, $p = 0.013$) with the low KUE plants containing significantly more Na in the polyhalite treatment than the high KUE plants. Additionally, within the high KUE grouping there was a significantly lower root Na content in the polyhalite treatment than in the AY2 treatment (Tukey's range test, $p = 0.031$).

At the flowering stage, significant interactions were found in the content of root K (Kruskal Wallis, $p = 1.4 \times 10^{-4}$) where the low KUE grouping had higher root K in both treatments than the high KUE grouping (Tukey's range test, AY2 high KUE – low KUE $p = 0.0054$, Poly high KUE – low KUE $p = 0.023$). Root P content also had a significant interactive effect between treatment and KUE (Kruskal Wallis, $p = 3.98 \times 10^{-4}$) with the low KUE grouping having a higher P content in the poly treatment than the high KUE grouping (Dunn's post hoc test, $p = 0.0035$). Panicle S content was also found to have a significant interactive effect (type

3 two-way ANOVA, p = 0.008) with the low KUE grouping having higher S content in the AY2 treatment than the high KUE grouping (Tukey's range test, p = 0.01).

Finally, at the grain sampling stage a significant interaction was seen in the shoot content of K (Kruskal Wallis $p = 8.4 \times 10^{5}$) with the low KUE grouping having higher K content in the AY2 treatment than the high KUE grouping (Dunn's post hoc test, $p = 8.39 \times 10^{5}$). A significant interactive effect was also seen in shoot S (type 3 two-way ANOVA, p = 0.008) however adjustment of p – values in the post-hoc test due to multiple testing led to no significant interactions being identified, the closest being found in the two KUE groupings response to the AY2 treatment where Tukey's range test showed a p-value of 0.092. Both total macronutrient shoot content per dry weight and overall nutrient shoot content per dry weight saw a significant interactive effect (type 3 two-way ANOVA, $p = 9.6 \times 10^{-4}$ and $p = 9.06 \times 10^{-4}$) with both finding a significant difference in the response of the high and low KUE groupings to the AY2 treatment. In both cases the low KUE cultivars had significantly higher total macronutrient and overall nutrient shoot content per dry weight than the high KUE cultivars. Significant interactive effects were also observed in panicle Ca content (Kruskal Wallis, p = 0.009) and panicle K content (log transformed type 3 two-way ANOVA, $p < 0.001$) at the grain sampling stage. However, in both of these cases the interaction was not of interest to this study with neither the different KUE groupings having significantly different responses to each other or to the treatments nor the individual KUE groupings having significantly different responses to the treatments.

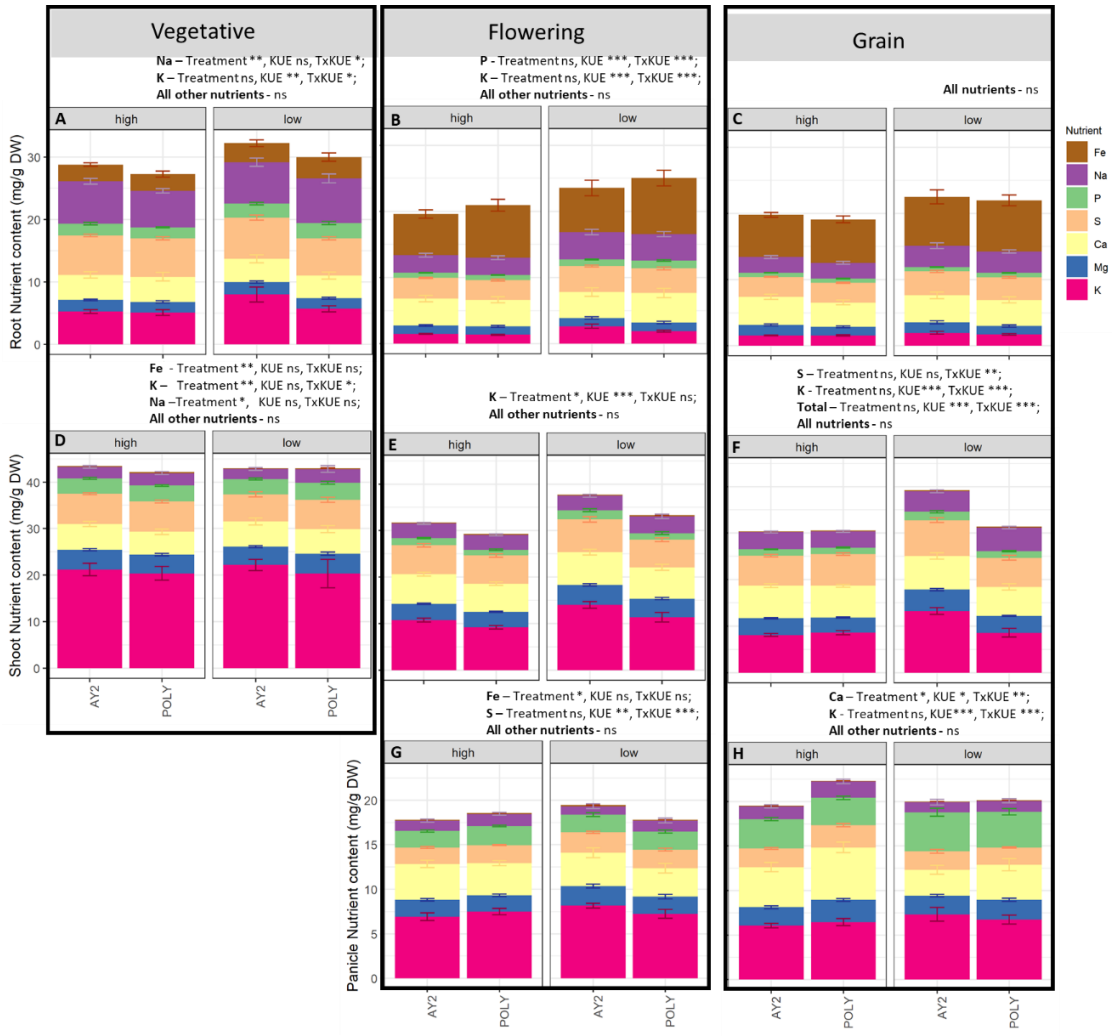


Figure 3.11 – Macronutrient and Fe content of rice cultivars grouped by KUE. Stacked barplots of nutrient content of roots (A, B and C), shoots (D, E and F) and panicles (G and H) panicles at three sampling stage. Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. High and low KUE cultivars are grouped into plots. Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) seen in the nutrient contents between treatments, cultivar (CV) and interactive effects (TxCV) are noted by asterisks in the top right corner above each panel of cultivar plots ((* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$). Each nutrient barplot represents the mean with error bars denoting the standard error.

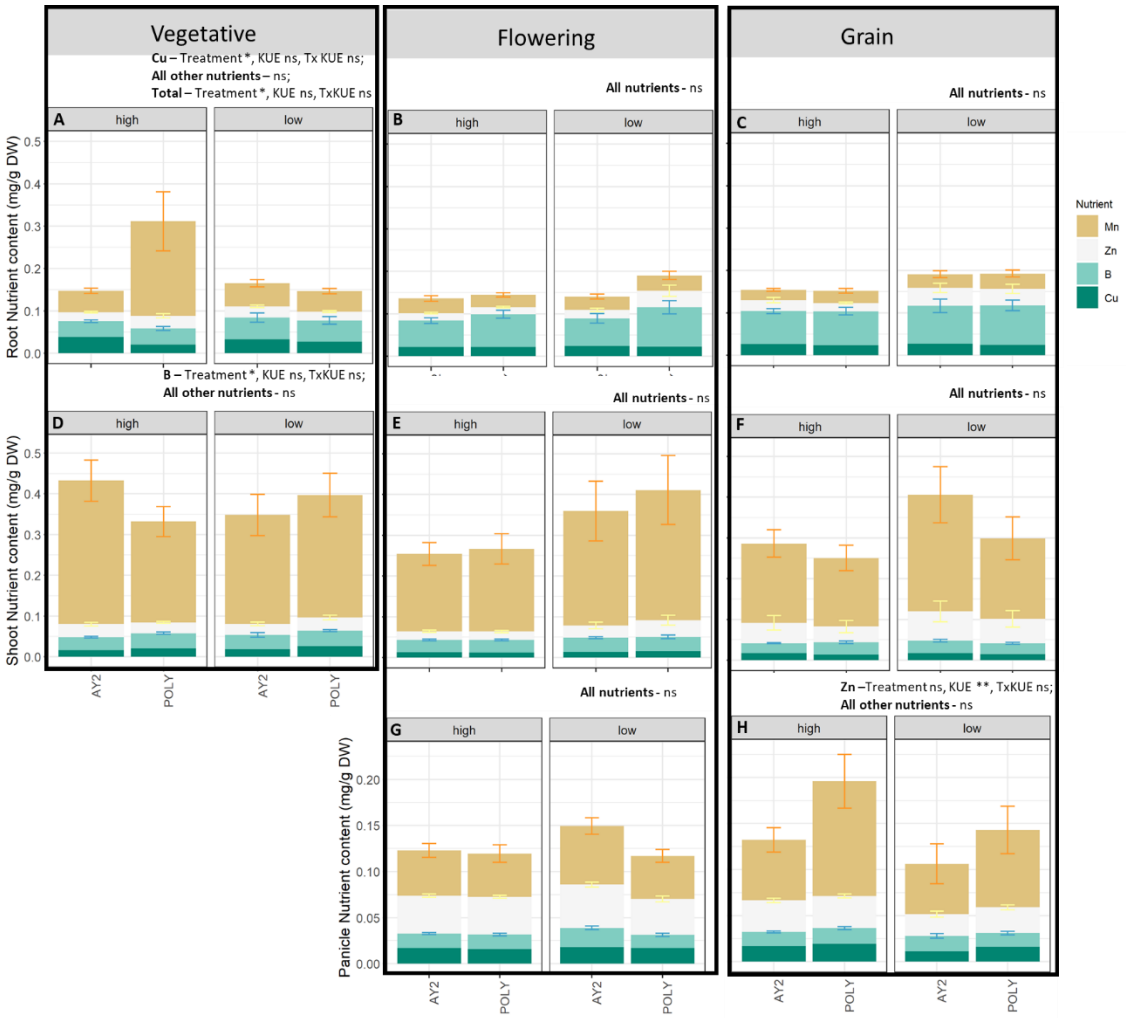


Figure 3.12 – Micronutrient content of rice cultivars grouped by KUE. Stacked barplots of nutrient content of roots (A, B and C), shoots (D, E and F) and panicles (G and H) panicles at three sampling stage. Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. High and low KUE cultivars are grouped into plots. Plants were grown with an adjusted yoshida (AY2) or polyhalite powder (Poly) treatment. Significant differences ($p < 0.05$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) seen in the nutrient contents between treatments, cultivar (CV) and interactive effects (TxCV) are noted by asterisks in the top right corner above each panel of cultivar plots ((* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$). Each nutrient barplot represents the mean with error bars denoting the standard error.

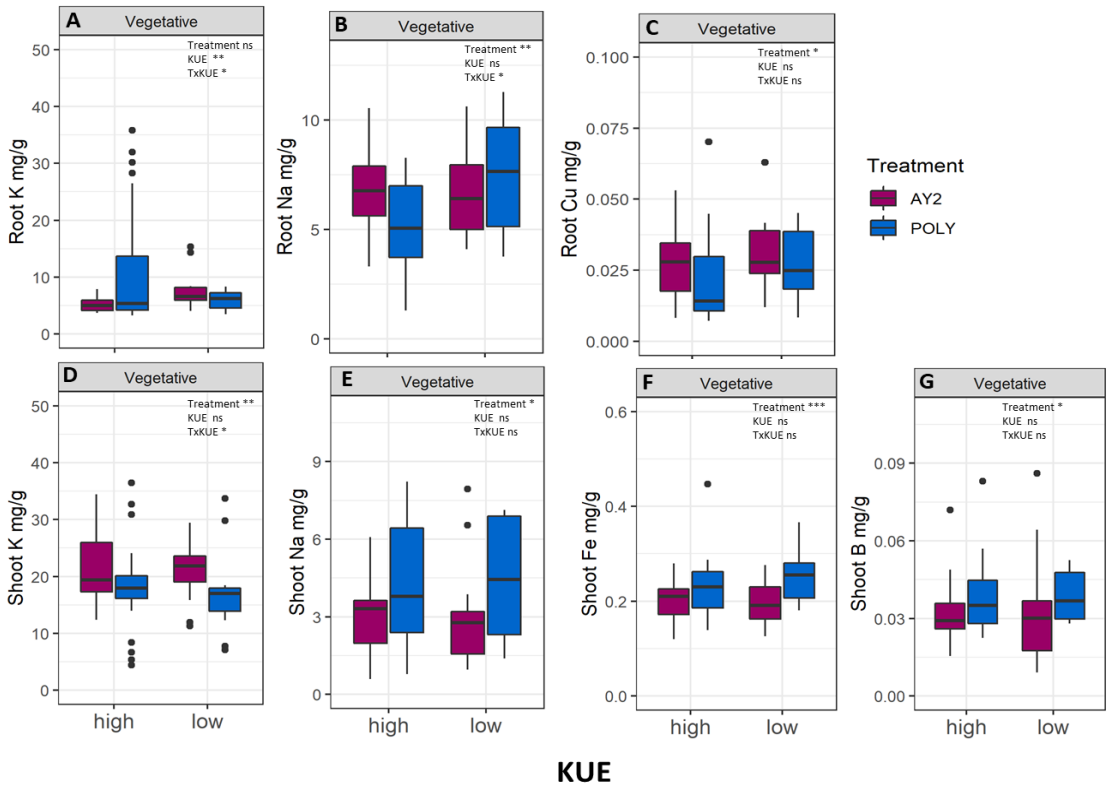


Figure 3.13 - Individual nutrient contents at the vegetative stage with significant differences of cultivars grouped by KUE. Boxplots of root K (A), Na (B), Cu (C) and shoot K (D), Na (E), Fe (F) and B (G). These nutrients had significantly different nutrient content between treatments or interaction between treatment and KUE (TxKUE) of plants grown with an adjusted yoshida (AY2) and polyhalite powder (Poly) treatment. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, KUE or interaction between treatment and KUE (TxKUE) are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

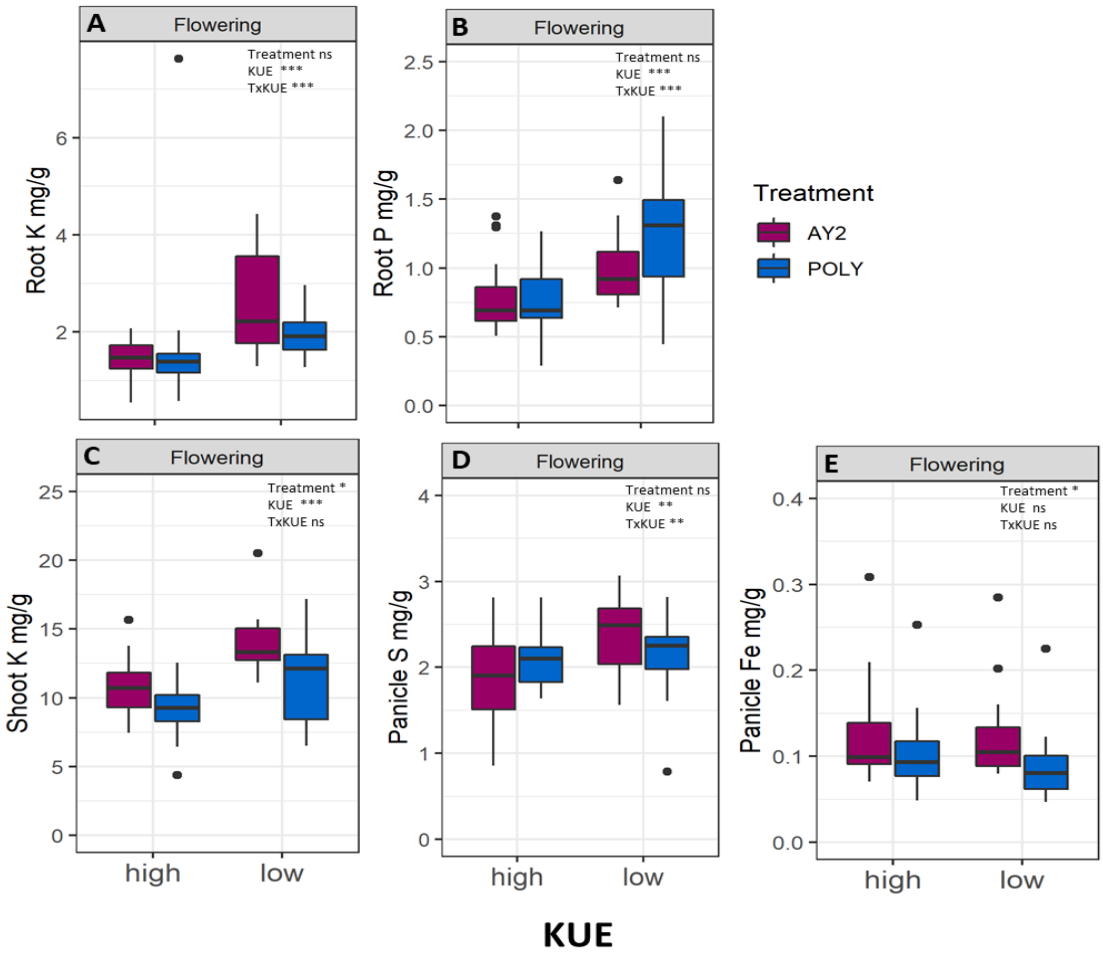


Figure 3.14 - Individual nutrient contents at the flowering stage with significant differences of cultivars grouped by KUE. Boxplots of root K (A) and P (B) and shoot K (C), S (D), N and Fe (E). These nutrients had significantly different nutrient content between treatments or interaction between treatment and KUE (TxKUE) of plants grown with an adjusted yoshida (AY2) and polyhalite powder (Poly) treatment. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, KUE or Interaction between treatment and KUE (TxKUE) are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

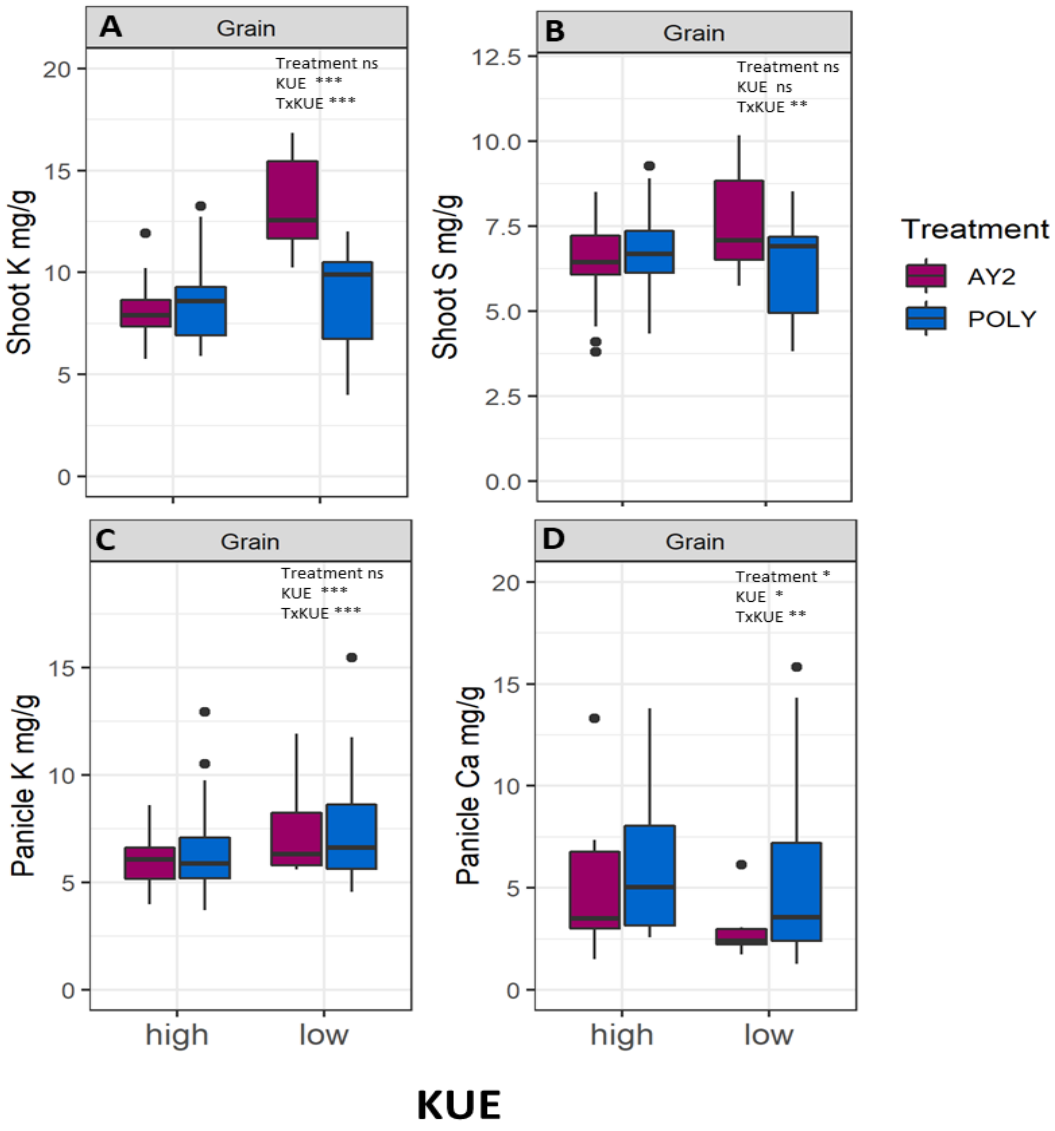


Figure 3.15 - Individual nutrient contents at the grain stage with significant differences of cultivars grouped by KUE. Boxplots of shoot K (A) and S (B) and panicle K (C) and Ca (D). These nutrients had significantly different nutrient content between treatments or interaction between treatment and KUE (TxKUE) of plants grown with an adjusted yoshida (AY2) and polyhalite powder (Poly) treatment. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, KUE or interaction (TxKUE) between treatment and KUE are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

Table 3.5 – Mean root nutrient contents and p-values for low and high KUE cultivars at the vegetative sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

	Root nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	8.89 a	2.08 a	6.64 a	4.14 a	2.41 a	7.24 ac	3.034 a	0.057 a	0.484 a	0.231 a	0.037 a
AY2 high KUE	5.40 ab	1.88 a	6.31 a	4.14 a	1.93 a	6.98 ab	2.643 a	0.038 a	0.093 a	0.070 a	0.022 a
Poly low KUE	5.70 ab	1.71 a	5.96 a	3.62 a	2.44 a	7.13 b	3.504 a	0.050 a	0.027 a	0.048 a	0.021 a
Poly high KUE	5.10 b	1.71 a	6.15 a	4.03 a	1.74 a	5.84 c	2.193 a	0.038 a	0.021 a	0.223 a	0.030 a
P-values											
Treatment	ns	ns	ns	ns	ns	0.007	ns	ns	0.042	ns	ns
KUE	0.0096	ns	ns	ns	ns	ns	ns	0.005	ns	ns	ns
Interaction (TxKUE)	0.032	ns	ns	ns	ns	0.033	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Means which had significantly different interactive effects are followed by different letters $p < 0.05$ with Two-way ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test

Table 3.6 – Mean shoot nutrient contents and p-values for low and high KUE cultivars at the vegetative sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

	Shoot nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	22.20 a	3.93 a	5.84 a	5.42 a	3.24 a	2.18 a	0.731 a	0.035 a	0.019 a	0.268 a	0.027 a
AY2 high KUE	21.21 a	4.25 a	6.50 a	5.52 a	3.30 a	2.45 a	0.227 a	0.032 a	0.016 a	0.352 a	0.032 a
Poly low KUE	20.35 a	4.27 a	6.29 a	5.29 a	3.66 a	2.93 a	0.950 a	0.038 a	0.026 a	0.301 a	0.033 a
Poly high KUE	20.39 a	4.02 a	6.51 a	4.90 a	3.44 a	2.66 a	0.898 a	0.038 a	0.020 a	0.248 a	0.026 a
P-values											
Treatment	0.0042	ns	ns	ns	ns	0.027	<0.001	0.041	ns	ns	ns
KUE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interaction (TxKUE)	0.0	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Means which had significantly different interactive effects are followed by different letters $p < 0.05$ with Two-way ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test

Table 3.7 – Mean root nutrient contents and p-values for low and high KUE cultivars at the flowering sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

Flower	Root nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	2.63 acd	1.26 a	3.92 a	3.92 a	1.01 ab	4.13 a	6.680 a	0.065 a	0.024 a	0.031 a	0.020 a
AY2 high KUE	1.47 bcd	1.27 a	3.13 a	4.08 a	0.78 ab	2.66 a	6.218 a	0.062 a	0.022 a	0.035 a	0.018 a
Poly low KUE	1.93 abc	1.25 a	3.66 a	4.30 a	1.28 a	3.84 a	10.027 a	0.092 a	0.023 a	0.036 a	0.039 a
Poly high KUE	1.62 abd	1.38 a	3.15 a	4.26 a	0.77 b	2.66 a	7.745 a	0.076 a	0.050 a	0.040 a	0.019 a
P-values											
Treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
KUE	<0.001	ns	<0.001	ns	<0.001	0.017	ns	ns	ns	ns	ns
Interaction (TxKUE)	<0.001	ns	ns	ns	<0.001	ns	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Means which had significantly different interactive effects are followed by different letters $p < 0.05$ with Two-way ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test

Table 3.8 – Mean shoot nutrient contents and p-values for low and high KUE cultivars at the flowering sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

	Shoot nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	14.03	4.28	7.03	7.06	1.97	3.10	0.231	0.035	0.013	0.281	0.030
AY2 high KUE	10.78	3.47	6.21	6.39	1.54	3.14	0.208	0.030	0.013	0.190	0.021
Poly low KUE	11.40	3.97	5.93	6.72	1.40	3.64	0.230	0.035	0.015	0.319	0.041
Poly high KUE	9.11	3.35	6.04	6.12	1.23	3.22	0.331	0.031	0.013	0.203	0.021
P-values											
Treatment	0.013	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
KUE	<0.001	0.001	ns	ns	0.041	ns	ns	ns	ns	0.023	ns
Interaction (TxKUE)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Table 3.9 – Mean panicle nutrient contents and p-values for low and high KUE cultivars at the flowering sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

	Panicle nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	8.28 a	2.17 a	2.37 a	3.69 a	2.07 a	0.91 a	0.187 a	0.021 a	0.018 a	0.064 a	0.047 a
AY2 high KUE	6.95 a	1.89 a	1.85 b	4.00 a	1.86 a	1.13 a	0.151 a	0.016 a	0.017 a	0.049 a	0.041 a
Poly low KUE	7.25 a	1.93 a	2.09 ab	3.19 a	2.04 a	1.23 a	0.093 a	0.014 a	0.017 a	0.047 a	0.039 a
Poly high KUE	7.50 a	1.86 a	2.06 ab	3.74 a	3.28 a	1.38 a	0.102 a	0.016 a	0.016 a	0.047 a	0.041 a
P-values											
Treatment	ns	ns	ns	ns	ns	ns	0.03	ns	ns	ns	ns
KUE	0.042	ns	0.0049	ns	ns	ns	ns	0.029	ns	ns	ns
Interaction (TxKUE)	ns	ns	0.008	ns	ns	ns	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Means which had significantly different interactive effects are followed by different letters $p < 0.05$ with Two-way ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test

Table 3.10 – Mean root nutrient contents and p-values for low and high KUE cultivars at the grain sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

	Root nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	1.94	1.59	3.61	4.08	0.65	3.23	7.382	0.089	0.028	0.032	0.042
AY2 high KUE	1.55	1.60	3.01	4.25	0.63	2.37	6.385	0.075	0.027	0.024	0.050
Poly low KUE	1.71	1.27	3.41	3.94	0.69	3.22	7.733	0.090	0.024	0.034	0.054
Poly high KUE	1.54	1.32	2.96	3.69	0.64	2.39	6.569	0.079	0.024	0.028	0.030
P-values											
Treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
KUE	ns	ns	0.002	ns	ns	0.0056	ns	ns	ns	ns	0.036
Interaction (TxKUE)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Table 3.11 – Mean shoot nutrient contents and p-values for low and high KUE cultivars at the grain sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

	Shoot nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	13.26 a	4.60 a	7.65 a	7.26 a	1.83 a	4.37 a	0.245 a	0.031 a	0.017 a	0.286 a	0.071 a
AY2 high KUE	8.13 b	3.60 a	6.43 a	6.98 a	1.37 a	3.74 a	0.157 a	0.024 a	0.017 a	0.195 a	0.050 a
Poly low KUE	8.60 b	3.63 a	6.27 a	6.21 a	1.43 a	5.06 a	0.213 a	0.027 a	0.014 a	0.198 a	0.060 a
Poly high KUE	8.61 ab	3.27 a	6.82 a	6.81 a	1.37 a	3.49 a	0.192 a	0.030 a	0.014 a	0.168 a	0.038 a
P-values											
Treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
KUE	<0.001	ns	ns	ns	ns	ns	ns	ns	ns	0.017	0.014
Interaction (TxKUE)	<0.01	ns	0.009	ns	ns	ns	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Means which had significantly different interactive effects are followed by different letters $p < 0.05$ with Two-way ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test

Table 3.12 – Mean panicle nutrient contents and p-values for low and high KUE cultivars at the grain sampling stage. p-values from 2-way ANOVA and Kruskal Wallis in response to different K fertiliser regimes

	Panicle nutrient content mg g^{-1}										
Treatment	**K**	**Mg**	**S**	**Ca**	**P**	**Na**	**Fe**	**B**	**Cu**	**Mn**	**Zn**
AY2 low KUE	7.32 a	2.11 a	2.08 a	2.90 a	4.38 a	1.14 a	0.057 a	0.017 a	0.011 a	0.055 a	0.024 a
AY2 high KUE	6.06 a	2.06 a	2.09 a	4.51 ab	3.30 a	1.42 a	0.068 a	0.015 a	0.017 a	0.063 a	0.037 a
Poly low KUE	7.73 a	2.71 a	2.71 a	5.53 ab	3.81 a	1.94 a	0.107 a	0.017 a	0.017 a	0.145 a	0.031 a
Poly high KUE	6.45 a	2.50 a	2.49 a	5.90 b	3.09 a	1.82 a	0.081 a	0.017 a	0.020 a	0.121 a	0.039 a
P-values											
Treatment	ns	ns	ns	0.042	ns	ns	ns	ns	ns	ns	ns
KUE	<0.001	ns	ns	0.017	0.006	ns	ns	ns	ns	ns	0.003
Interaction (TxKUE)	<0.001	ns	ns	0.01	ns	ns	ns	ns	ns	ns	ns

ns: non-significant

AY2: adjusted yoshida, Poly: polyhalite

Means which had significantly different interactive effects are followed by different letters $p < 0.05$ with Two-way ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test

3.3.3 Investigating the impact of chloride ions of rice growth and nutrient content in different rice cultivars

3.3.3.1 Growth analyses

A range of rice cultivars was grown in hydroponics with treatments of four different nutrient solutions based on yoshida with chloride concentrations of 0 mM, 2 mM, 3 mM and 6 mM. No significant differences were seen between the root or shoot dry weights for any of the cultivars in any of the treatments (fig. 3.16).

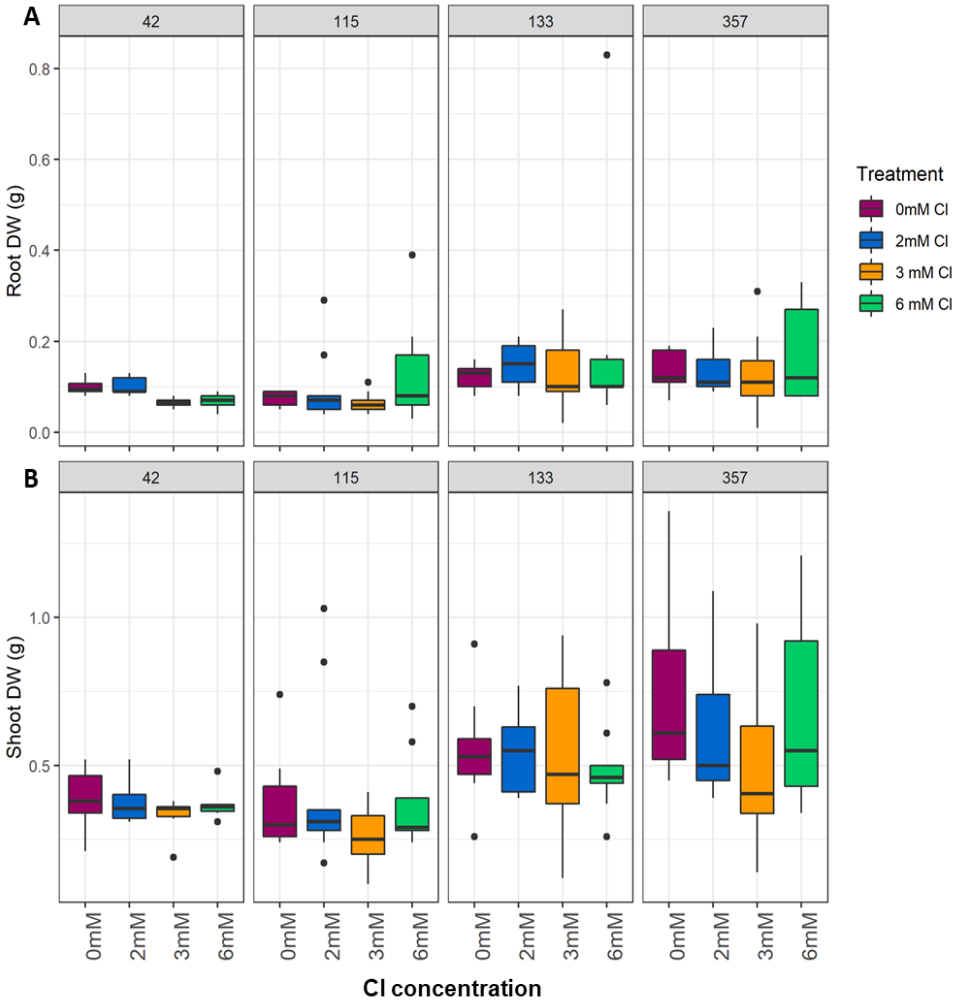


Figure 3.16 – Dry weights of four different rice cultivars grown in hydroponics with nutrient solutions containing differing Cl content. Boxplots of dry weights of roots (A), and shoots (B) of four different rice cultivars (identified along top of plot) grown in hydroponic solutions containing different Cl contents (0 mM, 2 mM, 3 mM, 6 mM). No significant differences were found between treatments, Kruskal Wallis test ($p < 0.05$).

3.4 Discussion

3.4.1 Investigating the use of polyhalite as a potassium fertiliser in sand with a range of rice cultivars

3.4.1.1 Growth analyses

Rice is an essential food source which has thousands of different cultivars and within this extensive range of genotypes there is wide variation in nutrient use efficiency. Due to the global importance of this crop it is important to determine how a range of rice cultivars responds to different K fertiliser types, especially whether these fertiliser sources have any differing effect on plant growth or crop yield. Few studies can be found in the literature that compare cultivar response to different K sources, comparisons of different K sources in one cultivar being more common. Studies which compare KCl and K_2SO_4 on the yield of one rice cultivar (Ghaffar et al., 1999; Mohd Zain & Ismail, 2016) found no significant differences between grain yields which is similar to results found in this study; where no significant difference in dry weights was found between treatments (fig. 3.1). These results are also similar to those found in maize where no significant differences in yield were found between polyhalite and other K fertiliser treatments (Dal Molin et al., 2020). There are a number of benefits to a lack of cultivar responses to specific K fertiliser sources. These include, farmers being able to use the best variety for their situation rather than having to determine the best cultivar - fertiliser relationship for their land. Additionally, breeders can focus solely on improving crop KUE without the added complication of matching cultivars with fertiliser sources.

This study only compared two different fertilisers in solution and therefore further studies could include polyhalite in the granule form to help establish whether the slow release nature of this fertiliser impacts rice cultivars differently, although the data from chapter 2 section 2.3.3 using an unbalanced fertiliser regime on just one cultivar, suggests that no difference in plant growth will occur, although TGW may differ between treatments.

3.4.1.2 Nutrient content

Given the importance of rice as staple food for many people, its nutritional content is very important. With a range of cultivars grown in different areas, it is crucial to evaluate

whether the nutrient content of different rice varieties alters when provided with different K sources.

Whilst collectively the cultivars were found to have significant variability in the content of some nutrients in this study, these were most likely due to inherent differences between cultivars; no significant interaction of cultivar and fertiliser treatment was found except in the grain shoot stage where overall total nutrient and total macronutrient contents both had a significant interactive effect between cultivar and treatment (fig. 3.3). The significant interaction in both of these measurements mostly showed differences between the large overall nutrient totals in cultivars 115 and 377 when grown with AY2 compared to the totals in other cultivars, whereas none of the within cultivar responses to treatment were significant. Different rice cultivars had varying levels of nutrient content (see appendix figs. 6.2.1 – 6.2.11), however the individual cultivars did not respond differently to the two K treatments. Nevertheless, future work to investigate whether rice cultivars utilise unbalanced fertiliser regimes (e.g. KCl and K2SO4 treatments), differently could be beneficial to farmers.

The overall average response of the cultivars had significant differences in a number of nutrients. Lower shoot K was found in the polyhalite treatment at the vegetative and flowering stage (figs. 3.3, 3.8 and 3.9). Not only are these results dissimilar to those found in tomatoes where foliar K was found to be higher in the polyhalite treatment than all other treatments (da Costa Mello et al., 2018b), it is also contradictory to results found in the previous chapter, where no significant differences were observed in the K content of any plant part between the polyhalite and AY treatments. The rice cultivar used in chapter 2 was not included within this set of cultivars and so may have had a different response to those in this chapter. Figures 3.3, 3.8, 3.9 and appendix figure 6.2.1 show that, whilst not significant different, at the vegetative stage the cultivars 357, 377 and 401 had lower K content in the polyhalite treatment compared to the AY2 treatment, whereas the other cultivars had no difference in K content between the treatments. The absence of a growth or yield penalty in the cultivars that could be associated with the lower shoot K content at these stages suggests that this reduction may not be detrimental to the plant, despite K's wide-ranging roles within the plant, however further experimentation would be advantageous to clarify this point. Unlike some other nutrients, K is only taken up in the ionic form. Therefore, whilst cultivar differences in N uptake may be

specific for the different forms of the nutrient e.g. ammonia versus nitrate (Hu et al., 2015), the K uptake transporters are attuned to K ions. The main differential factors between K fertiliser sources therefore are their accompanying anion and their dissolution rate.

Compared to a range of other plant based seed products including cereal groats, pulses and nuts, rice has the second lowest Ca content (Suliburska & Krejpcio, 2014). Calcium is not only essential for plant growth but also for humans, being necessary for bone and teeth structure and development as well as having a protective effect against many diseases including cardiovascular diseases and various types of cancer (Knez & Stangoulis, 2021). Biofortification of staple crops with Ca is a viable option for sufficient provision of Ca to populations who depend on plant foods as their main dietary intake. This strategy could be used in combination with fertilisers which improve grain Ca contents. Whilst the results of this study showed an average increase in panicle Ca content at the grain stage across the different cultivars with the polyhalite treatment (figs. 3.4 and 3.9), no similar results have been found in other studies. Despite increased foliage Ca in tomatoes provided with polyhalite being reported in one study, no accompanied increase in fruit Ca content was observed (da Costa Mello et al., 2020). Furthermore, the increased Ca result is not consistent with those found in the previous chapter, thus suggesting that further work is needed to clarify these results.

Lower shoot Mg content in the polyhalite treated plants was not sufficient to affect crop yields compared to the AY2 treated plants (figs. 3.3 and 3.9). Magnesium plays a pivotal role in chlorophyll synthesis (Cakmak and Kirkby, 2008), however at the time of grain sampling the plants had senesced, thus reducing the impact that lower Mg may have had on these organelles. As the lower Mg content was not seen at earlier lifecycle stages, nor did it appear to impact on plant dry weights, it is unlikely to be a concern except in Mg limiting conditions. This result was also not consistent with previous studies with both studies in tea (Zhou et al., 2019) and tomatoes (da Costa Mello et al., 2020) finding higher leaf Mg content in polyhalite treated crops.

No significant differences were seen in the overall cultivar S content in any plant part or sampling stage (figs. 3.2 – 3.4 and appendix fig. 6.2.4). These results are in agreement with those found in maize and the data presented in sections 2.3.2 and 2.3.3. Given the current rise

in global soil S deficiency it would be pertinent for future experiments to compare different cultivar reponses in an unbalanced fertiliser setting such as in section 2.3.3.

The lower panicle Fe content of the polyhalite treated plants (figs. 3.4 and 3.9) is not apparent in the later grain sampling stages which suggests that the polyhalite treated plants were slower in remobilising Fe into the panicle. During flowering and grain filling many nutrients are remobilized to allow for the production of new organelles and for seed filling. Whilst the difference in these plants was not detrimental to grain Fe content, variable environmental conditions in a field may exacerbate the timing of this nutrient movement. Seed Fe is not only important for human health, but also in the embryogenesis of seeds (Grillet et al., 2014) therefore if these low concentrations were continued to the ripe grain stage in a field setting it could affect seed development.

The higher shoot Fe found in the polyhalite treatment at the vegetative stage (figs. 3.3. and 3.8) is similar to results found at the seedling stage of the experiment in section 2.3.1, however it is not seen at later lifecycle stages. As discussed in the previous chapter, further analyses of nutrients beyond the four main macronutrients contained in polyhalite would be beneficial to a more extensive understanding of how it operates in comparison to other K fertilisers.

Sodium can act as a beneficial element in plants (Barker & Pilbeam, 2007), however in high concentrations can be detrimental to plant growth. The higher shoot Na content at the earliest sampling stage in the polyhalite samples (figs. 3.3 and 3.8) could become problematic if used on agricultural fields where salinity is a limiting factor. Sodium can also act to replace K as an osmoticum when K is scarce (Hartley et al., 2020). Polyhalite treated plants also had lower shoot K at this growth stage, therefore these plants could be utilising Na as a substitute until nutrient contents were redistributed later in the lifecycle of the plant.

As B plays an important role within the cell walls the higher shoot B at the vegetative stage in the polyhalite treated plants may mean they have slightly different cell wall structures to the AY2 treated plants (figs. 3.6 and 3.8). This elevated shoot B is only found at this sampling stage however suggesting that it is only a temporary change.

Pollution from urban development and mining wastewater can increase heavy metal contamination of paddy fields and is of increasing concern for human health (Cui et al., 2019). In a study of 38 rice cultivars Yan et al., (2006) found variable responses to increased Cu levels with increased, stable and decreased straw and grain yields being observed across the range of cultivars. Lower root Cu at the vegetative stage in the polyhalite treatment compared to AY2 treatment appears to be driven by the large differences seen between treatments in cultivars 401 and 357 (figs. 3.5 and 3.8), however, no significant interaction was seen between treatment and cultivar. Increased soil pH can cause Cu to bind more strongly to soil components therefore reducing Cu availability, whereas pH's at or below 6 provide adequate Cu availability (Barker & Pilbeam, 2007). However, as soil pH was not measured in this experiment it is difficult to ascertain if differences in the pH affected Cu availability. The low Cu in the polyhalite treatment at this early growth stage may be of benefit in polluted fields, although the change in Cu content between the treatments is only seen in the roots at the vegetative stage with no significant differences seen at later lifecycle stages. There is evidence that in soil with high Cu concentrations, rice plants sequester Cu in the root surface and epidermis rather than transporting it to the shoots (Cui et al., 2019) which helps explain why only root Cu differences were observed. Of all the parts of rice plants, the grains contain the lowest concentration of Cu (Yan et al., 2006) and in this study the grain content of both treatments fell within the maximum permissible limit of 10 mg kg^{-1} for human consumption (appendix figure 6.2.6).

3.4.2 Investigating the use of polyhalite as a potassium fertiliser in sand with a range of rice cultivars grouped by KUE

3.4.2.1 Growth analyses

The range of rice cultivars included in this experiment was chosen due to their high and low KUE capabilities in order to investigate whether how they affected a plant's ability to access nutrients from different K sources. Therefore, these cultivars were grouped by high and low KUE to enable analysis of the growth and nutrient contents of these two categories. Cultivars were initially chosen from previous work calculating KUE (SUE – table 3.4) by the RGR divided by the shoot K content of plants (Hartley et al., 2020). This measurement is determined by high growth rates with low shoot K, however it does not take root biomass into account.

When the SUE for each cultivar was calculated for this study the cultivars did not maintain the low and high KUE values as found by Hartley et al 2020. Therefore, a different KUE measure was used for this study, whereby the total crop dry weight was divided by the total plant K content (DMNP). This KUE calculation took the total plant into consideration, helping to provide a wider picture of how different K fertilisers may affect cultivars both above and below ground. Between the treatments no significant differences in dry weights were seen in the overall average of all the cultivars (fig. 3.13). Significant differences were seen in the interaction of the KUE type and treatment type, however they reflected the differences in dry weights of plant parts between KUE categories. For example, the significant interactions seen at the grain sampling stage in the shoot dry weight was between the high KUE cultivars which had a higher average shoot dry weight across the two treatments than the low KUE cultivars. Whilst this suggests that the high KUE cultivars may be able to grow more efficiently than the low KUE cultivars, there was no evidence of within KUE category dry weight responses to the different K fertiliser regimes.

3.4.2.2 Nutrient content

Arguably the most interesting nutrients to investigate between the KUE grouped cultivars are that of K and Na. As aforementioned, Na can act as a substitute for K when plants are faced with low K conditions, thus affecting KUE (Hartley et al., 2020).

In this study, whilst there were significant differences in both the shoot K and Na contents at the vegetative stage (figs. 3.11 and 3.13), these differences were only between the treatments and not between the low and high KUE groupings, suggesting that KUE grouping was not affecting this response. The root Na content at the vegetative stage did have a significant interaction between the treatment and KUE groupings, with the high KUE cultivars having a significantly higher Na content in the AY2 treatment than the polyhalite treatment, which was not observed in the low KUE treated plants. The difference in root Na content in the cultivar 357 (appendix fig. 6.2.6) could be a major contributor to the change in Na content between treatments seen in the high KUE plants. Whilst there was no significant difference when comparing cultivar response to the different treatments, this cultivar does appear to have higher Na in the AY2 treatment compared to the polyhalite treatment.

Whilst the root K content at the vegetative stage was not significantly different between treatments (figs. 3.11 and 3.13), the high KUE cultivars followed the trend of the K content in the polyhalite treated plants being higher than those with the AY2 treatment. This suggests that within the high KUE cultivars the higher root Na content of the AY2 treated plants was due to a slightly lower K content, leading to Na being used as a temporary substitute. This substitution did not occur at later life stages nor did the dry weights of the root or shoot differ significantly suggesting that this substitution was only short-term.

At the flowering stage there were significant differences in the shoot K content between both the treatment and KUE types but with no interactive effect (figs. 3.11 and 3.14). At this later stage the low KUE cultivars had higher shoot K than the high KUE cultivars which is in keeping with their KUE categorisation, as low KUE cultivars require a higher K quantity to ensure healthy growth. At this sampling stage there was no change in the Na content between cultivars (fig. 3.14 and appendix fig. 6.2.6). These results suggest that cultivars with a different KUE are able to access K from different fertiliser sources equally well. However, at the grain stage the shoot K content of the low KUE cultivars was significantly higher in the AY2 treatment compared to the polyhalite treated plants (figs. 3.11 and 3.15), whereas no significant differences were seen in the high KUE cultivars in response to treatment type. As this significant difference between treatments in K shoot content of low KUE cultivars is only seen at a late lifecycle stage it likely presents few problems in agricultural settings, as K accumulation usually reaches its highest point at anthesis after which K is reallocated from the leaves to the seeds (White, 2013). No differences were seen in the panicle K content between the treatments in the low KUE plants (fig. 3.11 and appendix fig. 6.2.1), reinforcing this point. This change in K content in the low KUE cultivars between the treatments also appears to be the main contributing factor causing significant differences in the total macronutrient and overall nutrient shoot contents at the grain stage

Other nutrient content analyses show that grouping by KUE did not impact much on the results, although a higher number of interactions were seen between the treatment type and KUE groupings than when looking at cultivars individually. The significant interactions seen in root K and P content at the flowering stage, panicle S content at the flowering stage (figs. 3.11 and 3.14) and shoot S content at the grain stage (figs. 3.11 and 3.15) showed differences

between the low and high KUE cultivars uptake of nutrients. For example, the root K content at the flowering stage was higher in the low KUE cultivars for both treatments than the high KUE cultivars, however there was no significant difference within KUE categories between treatment types.

Interactions found in K root and shoot content at the vegetative stage (figs. 3.11 and 3.13) and panicle Ca and K content at the grain stage (figs. 3.11 and 3.15) were due to calculations that were not of use to this analysis e.g. high KUE cultivars treated with AY2 versus low KUE cultivars treated with polyhalite.

3.4.3 Investigating the impact of chloride ions of rice growth and nutrient content in different rice cultivars

3.4.3.1 Growth analyses

Many plant species are Cl sensitive and therefore the use of KCl as a fertiliser source can be detrimental to their growth. For example, cotton plants grown with either a K_2SO_4 and KCl were found to have a depreciated yield when grown with KCl (Pervez et al., 2004). Chloride ions can damage plants by causing changes to the shape and structure of chloroplasts as well as damaging root tissues (Borges et al., 2004). Rice plants have a range of resistances to Cl with indica species being more sensitive than japonica species (Teng et al., 2006). Applications of Cl containing fertilisers have been reported to increase the upper 30cm of soil from 0.25 mM to 0.73 mM (White & Broadley, 2001). Recommended K fertiliser rates for lowland rice growth range from 0 – 140 kg ha^{-1} however most countries tend to have recommended levels between 20 – 45 kg ha^{-1} (Bijay-Singh & Singh, 2017). The Cl concentrations used in this experiment were most similar to those that would be applied with a 0 – 45 kg ha^{-1} KCl application, therefore these represented the lower end of recommended K applications. This study found that in low concentrations, Cl had no effect on root or shoot growth irrespective of the cultivar used (fig. 3.16). Therefore, in conditions where salinity is not a limiting factor, KCl should not restrict plant growth. Future work could look at the use of different K fertiliser sources in combination with salt stress conditions to help determine how KCl may affect plant growth in these situations.

3.4.4 Overall discussion and conclusions

Different K fertiliser treatments did not alter plant growth traits between rice cultivars. Whilst there were overall nutrient content differences across all the cultivars collectively, no cultivar responded differently to another when provided with different K fertiliser regimes.

Grouping by KUE produced a small number of significant interactive effects between KUE category and treatment that were of interest to this study. At the vegetative stage the high KUE cultivars had different root Na content between the two treatments, at the flowering stage panicle S content in the low KUE cultivars contained higher S content in the AY2 treatment than the high KUE cultivars. Also at the flowering stage root P content in the low KUE cultivars was higher than the high KUE cultivars in the polyhalite and whilst this trend was also apparent in the AY2 treatment, it was not significantly different. Finally, at the grain stage, shoot K in the low KUE cultivars was significantly higher in the AY2 treatment than the polyhalite treatment. As the differences in Na and S contents were not observed at later lifecycle stages and did not affect plant dry weights it suggests these changes may not have much agronomical importance. Whilst shoot K content differed between the treatments at all stages it was only at the latest harvesting timepoint that the low KUE category had a significantly different response to the high KUE cultivars, at which point it was unlikely to impact on yield factors. As no differences were seen in panicle grain weight, it is unlikely that the changes in shoot K would impact on yield. Further work including a number of unbalanced fertiliser regimes as well as field trials would be beneficial in helping to determine how rice cultivars respond to K fertiliser sources.

The high number of variables (number of cultivars and treatments) produced difficulties in using consistent statistical tests across the parameters measured. These difficulties could be addressed by using a higher number of plants per replicate. However, this was not possible here due to limited time for growing and processing samples. Furthermore, problems with germination of some cultivars, also gave unbalanced numbers of low and high KUE cultivars which in future would preferably be balanced between the groupings. Another difficulty arose from growing two plants in the same pot which made sampling at the flowering and grain timepoints difficult as flowering of the two plants did not always coincide. Whilst this range of cultivars was chosen for its high and low KUE groupings, another set of groupings could add more to this area of research for example the inclusion of some traditional varieties

compared to some modern high yielding varieties to help evaluate the uptake abilities of rice cultivars and their ability to utilise different K sources.

Chapter 4: Investigating the effect of potassium fertilisers on soil microbial communities

4.1 Introduction

Soil microbial communities have many roles that affect soil health. Decomposition of organic matter, nutrient cycling, nutrient mobilisation from insoluble minerals, soil aggregation and improving soil structure are just a few of the activities provided by these communities. In addition to environmental abiotic and biotic factors affecting the community structures of soil microbial populations, anthropogenic factors such as crop rotation, no tillage practices, pesticide applications and fertiliser application can all influence the soil microbiota.

Fertilisation regimes which include organic forms of fertiliser e.g. manure or straw, have been shown to cause larger differences between soil microbial communities and higher soil microbial biomass than inorganic fertilisers (Li et al., 2017; Marschner et al., 2003; O'donnell et al., 2001; Gu et al., 2009). Zhang et al (2017) observed that in alkaline soils, fertiliser regime had no effect on bacterial richness or diversity, however in near-neutral and acidic soils, treatments that contained manure compared to those containing inorganic chemical fertiliser had higher bacterial richness and diversity. As well as increasing the soil organic carbon content – a potentially important microbial food source - organic residues can also increase soil pH due to the release of hydroxide ions throughout the decomposition process (Zhang et al., 2017).

Numerous studies have investigated the effect of long-term fertilisation regimes on soil microbial communities. Most studies conclude that inorganic chemical fertilisation has no significant effect on soil microbial community composition (Williams et al., 2013; Babin et al., 2019; Gu et al., 2009; Li et al., 2017; Marschner et al., 2003). Shorter term fertilisation trials also see little effect between inorganic fertiliser treatments, with larger differences seen in microbial communities between crop types (Zhang et al., 2019). When differences are seen, it is often difficult to determine the driving factors behind the change. Often, N application will lead to lower soil pH as the conversion of urea and ammonium to nitrate releases H^+ (Zhang et

al., 2017; Geisseler & Scow, 2014). Lower pH can hamper bacterial growth as the majority of species have an optimal range between pH 6-8, suitable for most proteins to function (Zhang et al., 2017; Ma et al., 2019). Multiple studies have shown that soils with a lower pH tend to have reduced bacterial diversity compared to soils closer to a neutral pH (Zhang et al., 2017; Geisseler & Scow, 2014). Furthermore, soil acidification can lead to deficiencies in P, K, Mg and Ca which may alter plant and microbial growth (Wu et al., 2019). Applications of ammonium can also prevent Ca, Mg, K and Na from binding to soil aggregates, causing alterations in the nutrition available to plants and microbes (Wu et al., 2019). Another important consideration is that increased root growth and exudation from healthier, fertilised plants may lead to reductions in soil microbial diversity due to the plant driven selection pressures on rhizosphere biota (Celestina et al., 2019; Grunert et al., 2019).

Agronomic practices can also have considerable effects on soil microbial biomass and species diversity. Reduced tillage has been reported to increase soil microbial biomass compared to conventional tillage practices (Berner et al., 2008) as it diminishes the negative impact of tilling on soil hyphal abundance and arbuscular mycorrhizal colonization (Kabir et al., 1997). However, other studies have shown that tillage practices have little impact on the composition of microbial phyla present (Babin et al., 2019).

The literature investigating slow release or controlled release fertiliser impact on soil microbial communities is limited and mostly focuses on N fertilisers. Calcium cyanamide, a synthetic slow-release N fertiliser, was found to significantly alter soil microbial community structure in celery plants compared to urea and a celery specific slow release fertiliser (Wu et al., 2019). At the harvesting timepoint, higher concentrations of Gram-positive bacteria and lower concentrations of Gram-negative bacteria and fungi were found in the calcium cyanamide treatment compared to the other treatments. Additionally, the soil microbial biomass C content increased from 414 – 633 µg C g^{-1} soil within two weeks of the calcium cyanamide application. However, the calcium cyanamide application also significantly altered the soil pH, whereas the other treatments had no effect on soil pH making it difficult to dissect the true cause of the community change. Another study investigating a polymer coated controlled release urea and conventional urea, found variable results between locations with no measurable effect of fertilisation application on the functional diversity of the bacterial

species being found in the majority of locations (Lupwayi et al., 2010). The resins and coatings which allow the functionality of slow or controlled release of urea have also been reported to have little impact on soil microbial biota (Pan et al., 2016).

Whilst studies in slow or controlled release fertilisers have shown little impact on soil microbial communities, most have concentrated on N fertilisers and there is limited research into how slow release K fertilisers may impact rhizosphere microbial species. Additionally, despite the importance of K fertilisers in agriculture and their extensive use globally, there is minimal literature on the effect of different K fertiliser sources on soil microbial communities. Studies that have investigated the impact of K fertilisers on soil microbial communities have only focussed on soil microbial activity (Ino & Monsi, 1964; Belay et al., 2002; Moro et al., 2014) with none investigating the impact of K fertilisers on microbial community species diversity or composition. Additionally, these previous studies have only focussed on KCl (Belay et al., 2002; Moro et al., 2014) or KNO_3 and KH_2PO_4 (Ino & Monsi, 1964) without considering K_2SO_4 or polyhalite. Therefore, we know nothing about the effect of polyhalite on the soil microbiome nor of the effect of K fertilisers in general on microbial community species composition and this research is a first attempt to understand these two factors.

Diversity of an ecological community can be measured using a variety of metrics, with alpha diversity metrics describing the diversity within a sample whereas beta diversity metrics express the diversity between different samples. The structure of an ecological community can differ in regard to its richness (number of taxonomic groups), evenness (the distribution of abundances of taxonomic groups) or a combination of both of these factors. The Simpson index of diversity (Simpson, 1949) is an alpha measure of diversity taking into account the richness and evenness of the species and predicts the likelihood of two individuals from a sample being of the same species. The original calculation of the Simpson's index of diversity (D) produces low values for communities with high diversity and high values for communities of low diversity. Therefore, this index is frequently transformed by either using the inverse 1/D or 1-D. In this work the Simpson index of diversity (1-D) was used to ensure that the values of the Simpson index increase with increasing diversity, rather than using the original formulation of D. With this transformation, values for D range between 0 and 1, where 1 represents infinite

diversity and 0 represents no diversity. The Simpson index of diversity (1-D) is calculated using the following calculation:

$$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$$

Where: n = total number of organisms of a taxonomic group
N = total number of organisms of all taxonomic groups

The Bray-Curtis dissimilarity (Bray & Curtis, 1957) is a beta diversity metric used to quantify the compositional differences in species populations between sites, or in this case, treatments. The Bray-Curtis dissimilarity is always a number between 0 and 1, where 1 means no species are shared between the communities with different treatments and where 0 means that communities with different treatments share all the same species. Bray-Curtis dissimilarity is calculated by:

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j}$$

Where: i and j are the two treatments

C_{ij} is the sum of only the lesser counts for each species found in both treatments

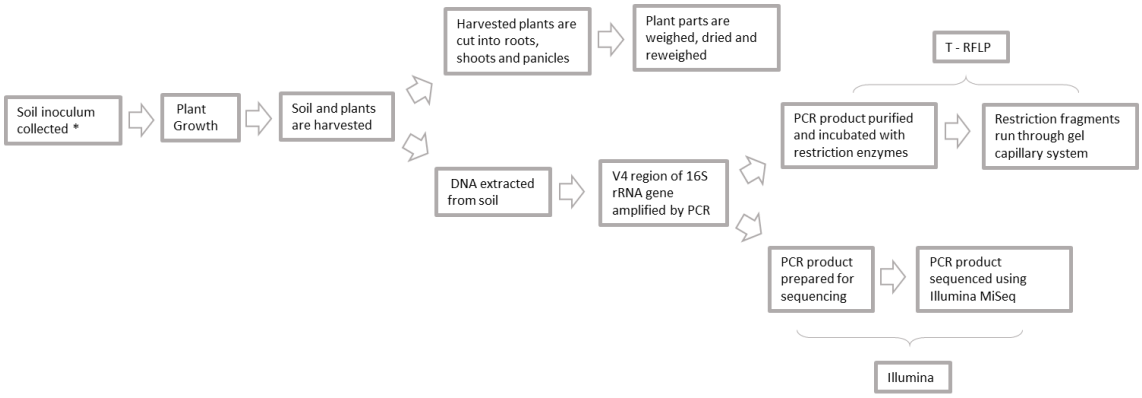
S_i is the total number of specimens counted at treatment i

S_j is the total number of specimens counted at treatment j

In this study the impact of an unbalanced and balanced K fertiliser regime on the soil microbial communities of rice plants grown in glasshouse conditions was observed. The balanced fertiliser regimes allowed observations of how different forms of polyhalite fertilisers impacted the soil microbial communities. In the balanced fertiliser regime microbial communities were comprised of species that had colonised the growth media from the glasshouse environment. Soil microbial communities were analysed using amplification of the V4 region of the 16S rRNA gene followed by production of Terminal Restriction Fragment Length Polymorphisms (T-RFLPs). Using T-RFLPs gives a broad picture of whether microbial communities contain different species between treatments however they give little depth as to the actual species composition within the soil microbial communities.

The unbalanced fertiliser regime provided the opportunity to evaluate how different K fertiliser sources affected the soil microbiota. In this experiment, soil inoculum was used to provide additional microbial species to the growth media. Illumina sequencing of the V4 region of the 16S rRNA gene were used to evaluate the soil microbial populations which provides more detail of the species composition of the soil microbial communities.

4.2 Materials and Methods



4.2.1 Investigating the effect of balanced K fertiliser regimes on microbial communities using T-RFLPs

4.2.1.1 Growth methods

Rice (*Oryza sativa cv. Zhenshan 2)* seeds were sown and germinated in sand with deionised water. After three weeks, 3 seedlings per treatment were transferred to 9 cm x 9 cm x 9 cm pots filled with 1.4 kg sand. Pots were placed in 14 cm diameter, 4 cm high plastic pots covered in black tape to reduce evaporation and algal growth. Plants were fed after transfer to pots and then subsequently every 2 weeks for 6 weeks and were arranged in a complete randomised block. Fertiliser treatments were; 1) an adjusted yoshida medium (AY2), 2) a polyhalite solution made from polyhalite powder (PP), 3) a polyhalite solution made with polyhalite granules (PGS) and 4) polyhalite granules applied to the surface of the sand (PG). All polyhalite containing treatments had additional N, P and micronutrients added either in the solution (PP and PGS) or poured onto the pot after granules were applied (PG) (table 2.3).

Plants were sampled at 10 days after final fertiliser addition (veg), after the first panicle fully emerged (flower) and when grain was fully ripe (grain). Plants were grown in a glasshouse with a 12 hour day/night cycle. Day temperatures were 28-32˚C and night temperatures were 24-30˚C. This growing procedure was repeated three times to reduce any seasonal effects (n = 27 plants per treatment).

4.2.1.2 Growth substrate sampling

Sand samples were taken by removing the plant and soil from the pot and shaking the excess sand from the roots of the plant. Once the main bulk of the sand was removed from the roots, they were placed in a plastic bag and shaken thoroughly to remove sand from the roots. A sample of this sand was placed into eppendorfs and flash frozen in liquid nitrogen. Samples were stored in a -80˚C freezer until they were freeze dried overnight. Once freeze dried, samples were stored in a plastic box with a small quantity of silica to prevent samples absorbing moisture from the air.

4.2.1.3 DNA extraction and T-RFLP methods

DNA was extracted from sand samples using a DNeasy PowerSoil Kit (Qiagen) following kit instructions with the following alterations: after addition of solution C5, tubes were incubated at room temperature for 5 minutes and 50 µl of solution C6 heated to 60°C was used instead of 100 µl. Samples from the same seasonal replicate were pooled according to treatment and harvest timepoint (n = 3 soil samples per pool). Extracted DNA was then amplified in a 50 µl PCR reaction using GoTaq G2 Flexi DNA polymerase (Promega) and primers 7F with a 5' hex fluorescent tag: 5' HEX AGA GTT TGA TYM TGG CTC AG and 806R primers: 5' GGA CTA CHV GGG TWT CTA AT 3'. PCRs were performed using 0.2 mM dNTPs, 0.4 µM of each primer, 0.625 units GoTaq G2 enzyme, and the manufacturers reaction buffer (Promega). The thermal PCR profile was as follows: initial denaturation for 2 min at 94°C followed by 40 cycles consisting of denaturation at 94°C for 30 s, primer annealing at 48°C for 45 s and elongation at 72°C for 1.5 min, the final elongation step was extended to 30 minutes. Amplification was performed in 0.2 ml reaction tubes in a DNA thermal cycler (BioRad T100). The PCR product was then purified using a NucleoSpin gel and PCR clean-up kit (Macherey-Nagel). Cleaned PCR products were digested with the restriction enzymes; Hha1, Rsa1 and BstU1 (NEB) in a 10 µl reaction composed of 100 ng DNA, 1 µl buffer, 1 unit of enzyme and sterilised nuclease-free

water to make the volume up to 10 µl. Further processing of samples was performed by the University of York's technological facility staff where samples were mixed with Hi-Di formamide (Thermofisher) and the size standard GeneScan 600 LIZ (Thermofisher) and were run through a 3130x1 Genetic Analyser capillary gel system.

4.2.1.4 Analyses of T-RFLP data

Data produced in the 3130x1 Genetic Analyser capillary gel system was transferred to the ThermoFisher Cloud, where the data was processed using the Peak Scanner CE Fragment Analysis app. Each T-RFLP run through the gel system was graphically represented as a peak, with its position representing its base pair (bp) length and peak height and area being determined by the fluorescent signal which is related to the quantity of the T-RF in the sample. Peaks smaller than 50 bp were removed to eliminate any background noise or primer dimer left in the sample. Data were then aligned using T-Align software (Smith et al., 2005). The data output from this software was normalised by dividing the area under each peak by the total peak area of each sample to account for run-to-run variations. Data from each restriction enzyme were then combined. A Bray-Curtis similarity matrix was constructed using the *vegan* R package (Oksanen et al., 2020) in R software (version 3.6.1, R Core Team, 2019) and visualised using an NMDS plot using the *ggplot2* package (Wickham, 2016).

4.2.1.5 Statistical analyses

The community composition between treatments and harvesting timepoints was compared using permutational multivariate analysis of variance (PERMANOVA, 999 permutations, Bray-Curtis distances) using the *adonis* function in the *vegan* package (Oksanen et al., 2020).

4.2.2 Investigating the effect of unbalanced K fertiliser regimes on microbial communities using Illumina sequencing

4.2.2.1 Soil collection for microbial inoculum

Soil was collected from two sites on the University of York campus on 27/10/20. Soil was collected from reed beds containing plants from the *Phragemites* and *Typha* genera, on Campus East and from the walled gardens site on Campus West which is representative of local agricultural soils. Approximately half of the soil from each site was autoclaved, whilst the rest of the collected soil was left overnight at room temperature.

A rice compost mix was made up using sand (10 %), vermiculite (15 %) and John Innes 2 (75 %) which was sterilised in the autoclave along with additional sand. A sterile soil mix was then made using 5 % sterilised reed bed soil, 5 % sterilised walled garden soil, 40 % sterilised rice compost mix and 50 % sterilised sand. An active microbial soil mix was also made up using, 5 % reed bed soil, 5 % walled garden soil, 40 % sterilised rice compost mix and 50 % sterilised sand. Soil samples were taken from both the sterile and active soil mixes prior to the experiment, placed into 50 ml falcon tubes and stored in a -80 ^{o}C freezer.

4.2.2.2 Growth methods

Rice (*Oryza sativa cv. Zhenshan 2)* seeds were sown and germinated in sand with deionised water. After two weeks 10 seedlings per treatment were transferred to 8 cm x 8 cm x 8 cm pots filled with either sterile or active soil mix. Pots were placed in Ø12 cm, 4 cm high plastic pots to allow for collection and reuse by plants of excess fertiliser treatments and water. Pots were arranged in a complete randomised block. Plants were fed after transfer to pots and then every 2 weeks for another four weeks. Fertiliser treatments were; 1) 200 ml deionised water only (No Fert) 2) no K fertiliser added (No K) 3) 200 ml adjusted yoshida (AY2), 4) polyhalite powder added to a pot (PP), 5) polyhalite granules added to a pot (PG) 6) KCl powder added to a pot (KCl). A 200 ml of solution containing N, P and micronutrients was added to treatments No K, PP, PG and KCl (table 4.1). Plants and soil were sampled at 14 days after final fertiliser addition. Day temperatures were 28-32 °C and night temperatures were 24-30 °C.

Table 4.1 – Nutrient concentrations for experiment 4.2.2.2

Nutrient	Chemical providing nutrient	Concentration of nutrients in No K medium (mM/l)	Concentration of nutrient in Poly Granules (PG and PP) medium (mM/l)	Concentration of nutrient in Adjusted Yoshida (AY2) medium (mM/l)	Concentration of nutrient in KCl medium (mM/L)
N	NH_4NO_3	14.5	14.5	14.5	14.5
P	$NaH_2PO_4.2H_2O$	1.5	1.5	1.5	1.5
K	Polyhalite (K_2O) K_2SO_4 KCl	- - -	5 - -	- 5 -	- - 5
Ca	Polyhalite (CaO, $CaSO_4$) $CaSO_4$ $CaCl_2.2H_2O$	- - -	5.5 - -	- 5 0.5	- - -
Mg	Polyhalite (MgO, $MgCO_3$) $MgSO_4.7H_2O$	- -	3 -	- 3	- -
S	Polyhalite (SO_3, $CaSO_4$) K_sSO_4 $CaSO_4$ $MgSO_4.7H_2O$	- - - -	10.5 - - -	- 2.5 5 3	- 2.5 - -
Na	Polyhalite (NaCl) $NaH_2PO_4.2H_2O$ Na_2SiO_3 NaCl	- - -	1 1.5 1.25 -	- 1.5 1.25 1	- - - -
Mn	$MnCl_2.4H_2O$	0.05	0.05	0.05	0.05
Mo	$(NH_4)_6.Mo_7O_{24}.4H_2O$	0.005	0.005	0.005	0.005
B	H_3BO_3	1	1	1	1
Zn	$ZnSO_4.7H_2O$	0.001	0.001	0.001	0.001
Cu	$CuSO_4.5H_2O$	0.001	0.001	0.001	0.001
Fe	$FeCl_2.6H_2O$	0.2	0.2	0.2	0.2

4.2.2.3 Growth analyses

Harvested shoots were weighed and dried in a fan oven at 80 °C for 72 hours, after which they were reweighed.

4.2.2.4 Soil sampling

Soil samples were taken as in 4.2.1.2.

4.2.2.5 DNA extraction and sequencing methods

A 1 µl spike-in of genomic DNA from *Thermus thermophilus* was added to each soil sample prior to DNA extraction at a concentration of 0.0081 ng/µl in accordance with Smets et al., 2016. DNA was extracted from soil samples as described above in *section 4.2.1.2.* Control samples were produced using the original sterile and active soil as well as DNA kit controls which used only the kit reagents. The 16S region of the extracted DNA was then amplified in a 25 µl PCR reaction using GoTaq G2 Flexi DNA polymerase (Promega) and primers with Illumina adapter sequences: 515F – GTGYCAGCMGCCGCGGTAA (Parada et al., 2016) and 806R – GGACTACHVGGGTWTCTAAT (Caporaso et al., 2012). PCRs were performed using 0.2 mM dNTPs, 0.4 µM of each primer, 0.625 units GoTaq G2 enzyme and the manufacturers reaction buffer (Promega). The thermal PCR profile was as follows: initial denaturation for 2 min at 94°C followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, primer annealing at 53°C for 45 seconds and elongation at 72°C for 1.5 min, the final elongation step was extended to 10 minutes. Amplification was performed in 0.2 µl reaction tubes in a DNA thermal cycler (BioRad T100). The PCR product was then purified using 0.8 X AMPure beads (Beckman Coulter). Cleaned PCR products were diluted with PCR grade water to between 4-10 ng/µl DNA. Further processing of samples was performed by the University of York's technological facility staff where, amplicons tagged with the Illumina adapter sequence were then subject to a final round of amplification to add unique barcode sequences. Eight cycles of PCR amplification were performed using NEBNext Q5 Polymerase 2X mastermix (New England Biolabs) and Illumina Nextera XT indexing primers. Amplicons were purified using 0.9 X AMPure XP beads (Beckman Coulter) and eluted into low TE buffer before quantification and pooling at approximately equimolar ratios. Samples were diluted to 4 nM before denaturing

with 0.2 M NaOH ready for sequencing at a final concentration of 12 pM, with a 20 % PhiX library spike in (Illumina; for added sequence variety). Amplicon pools were sequenced on an Illumina MiSeq using a v3 600 cycle kit, with paired end 300 cycle sequencing and dual 8 bp indexing reads, and the fastq generation workflow in Illumina's BaseSpace Sequence Hub.

4.2.2.6 Sequence data analysis

All bioinformatics analyses were performed in R (Version 4.0.2, R Core Team). Sequences generated from 16S amplicon libraries were demultiplexed and adapter and primer nucleotides were removed from the sequences. Forward read single-end amplicons were trimmed to a length of 200 bp and reverse read single-end amplicons were trimmed to a length of 160 bp, corresponding with a decline in the proportion of reads extending past this length. Reads were quality filtered (<2 errors per read), dereplicated and assigned to Amplicon Sequencing Variants (ASVs) using the *dada2* algorithm (Callahan et al., 2016) which clusters sequences with near 100 % similarity into ASVs. Chimeras were also removed using the *dada2* R package, resulting in 9004 ASVs. Taxonomy was assigned using a Bayesian classifier method as implemented by *dada2* using the SILVA database (Quast et al., 2013).

Taxonomic analysis of the 16S sequences was performed using the R package *phyloseq* (McMurdie & Holmes, 2013). Contaminating DNA sequences were identified using the *decontam* package (Davis et al., 2018) following the prevalence contamination method, resulting in 8484 ASVs remaining. ASVs were subsequently clustered into 97 % OTUs using the R package *DECIPHER* (Wright 2016) resulting in 5432 ASVs remaining. Mitochondrial and chloroplast sequences as well as ASVs not determined to the phylum level were then filtered out, leaving 4180 ASVs. Sequencing depth ranged from 5345 to 226361 reads. To normalise species abundances across samples with differing sequencing depth, read counts were converted to relative abundances. The *T. thermophilus* spike-ins were intended to be a reference point for soil bacterial abundances rather than using relative abundance measurements, however poor amplification of the genomic DNA meant this was not possible.

4.2.2.7 Community analysis

Alpha diversity was calculated for treatments using *phyloseq* using the Simpson index of diversity and compared using Kruskal-Wallis followed by Dunn's post-hoc test. The

community composition between treatments was compared visually using NMDS using the plot_ordination function in the *phyloseq* package and statistically using PERMANOVA (9999 permutations, Bray-Curtis distances). This was followed by a pairwise PERMANOVA using the R package *mctoolsr* (Leff, 2016) grouping samples by treatment and using the p-values corrected for multiple testing with false discovery rate (FDR) corrections. Significant differences in family relative abundance in the top twenty taxa between treatments were calculated using Kruskal Wallis followed by Dunn's post-hoc test ($p < 0.05$).

4.3 Results

4.3.1 Investigating the effect of balanced K fertiliser regimes on microbial communities using T-RFLPs

The effect of balanced K fertiliser sources on the composition of soil microbial communities was observed to determine whether there was an impact on species composition or distribution between different K fertiliser treatments. The relationships among rhizobacterial communities between different K fertiliser treatments were assessed using NMDS ordination. There was no discernible pattern of grouping of bacterial communities between treatment type when relative abundance data obtained by T-RFLP analysis was used (fig. 4.1). Samples were loosely clustered between sampling time points. Differences in the soil bacterial communities between treatments and sampling time points were statistically analysed using the non-parametric PERMANOVA test. No significant differences were seen in T-RFLP relative abundance between K fertiliser treatments (fig. 4.1), however the relative abundance at sample time point showed significant differences ($R^2 = 0.19$, $p < 0.001$) in the bacterial relative abundance between sampling times.

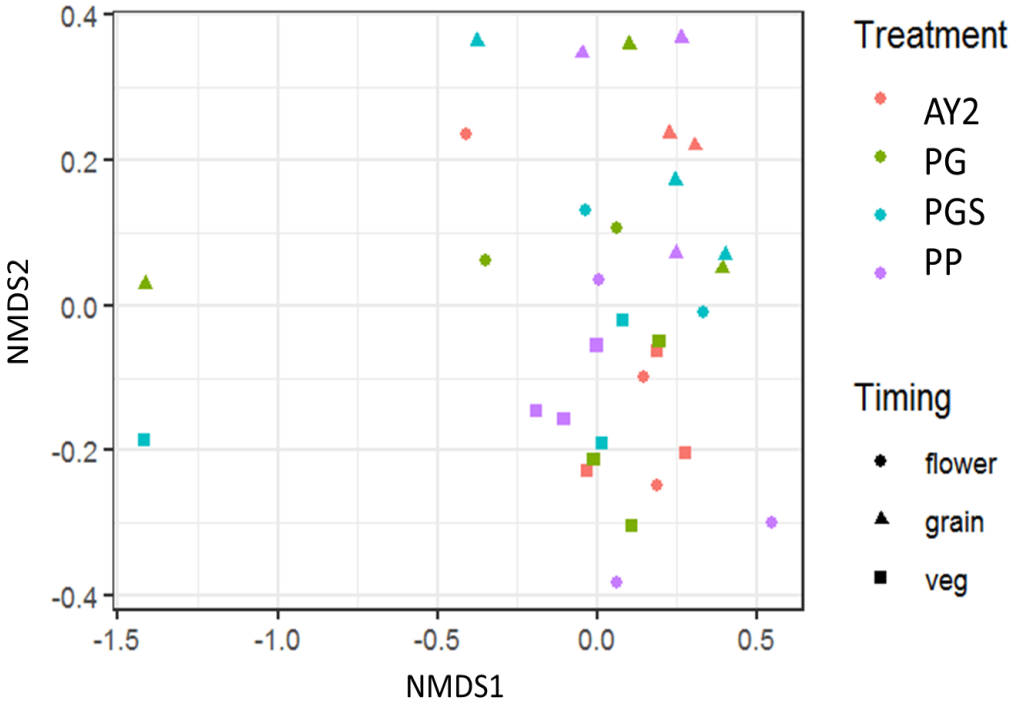


Figure 4.1- Soil bacterial community composition of plants grown with balanced K fertiliser regimes. NMDS plot of the bacterial community composition generated from the relative abundance Bray-Curtis matrix obtained using T-RFLPs in balanced fertiliser regime pots. Each point represents three pooled DNA samples from the same treatment and seasonal growing block. Different treatments included adjusted yoshida (AY2), polyhalite powder in solution (PP), polyhalite granules (PG) and polyhalite granules in solution (PGS). Plants were harvested at different lifecycle stages: vegetative (veg), first panicle emergence (flower) and 1 month after first panicle emergence (grain).

4.3.2 Investigating the effect of unbalanced K fertiliser regimes on microbial communities using 16s rRNA sequencing

4.3.2.1 Growth Analyses

The effect of different unbalanced K fertiliser treatments on a soil microbial inoculum was observed through sequencing of the V4 region of the 16S rRNA gene. As plants are able to influence the composition of the rhizosphere microbial communities, shoot growth (fig 4.2) was measured to observe how the plants had been affected by the different K treatments and to determine whether the inocula used had any negative impact on plant growth. No significant differences (two-way ANOVA, $p = 0.036$) were seen between plants grown on the active or sterile inoculum, however fertiliser treatment did significantly affect shoot dry weight (two-way ANOVA, $p = 2 \times 10^{-16}$). Plants grown with no fertilisation had significantly smaller shoot dry weights than all other treatments (Tukey's range test, $p < 0.05$). Whilst plants grown with no K fertilisation were larger than those with no fertiliser addition they still had a significantly lower shoot dry weight than all K containing treatments (Tukey's range test, $p < 0.05$). No significant differences (Tukey's range test, $p < 0.05$) were seen in shoot dry weight between the different K containing fertilisation regimes.

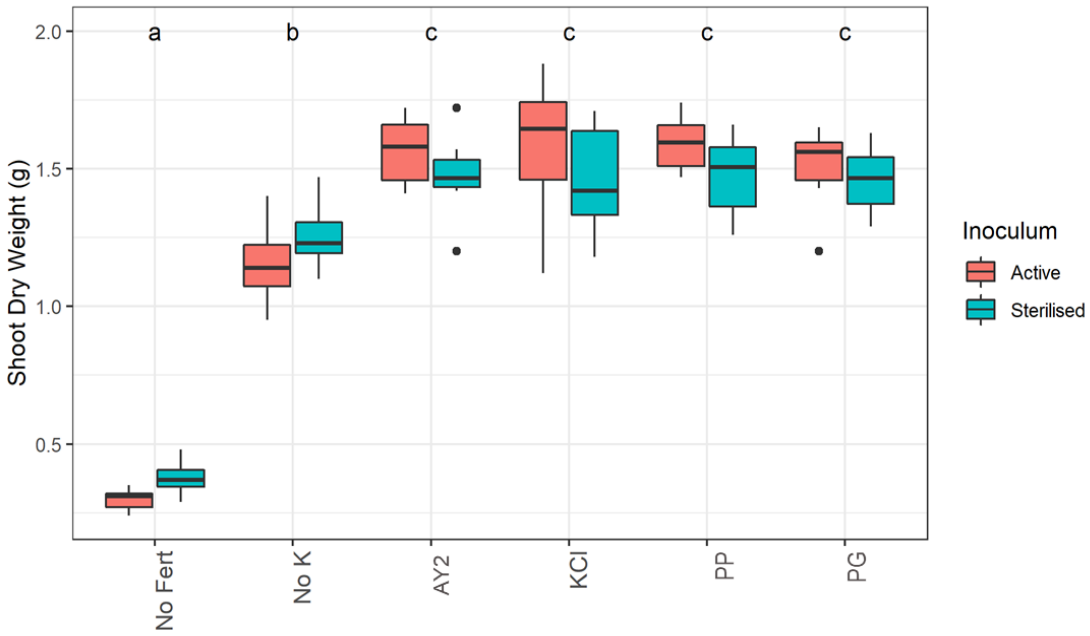


Figure 4.2 Shoot dry weights of plants grown with unbalanced K fertiliser regimes. Boxplots of shoot dry weights of plants grown in pots with no fertiliser (No Fert), no K fertiliser (No K), adjusted yoshida (AY2), KCl, polyhalite powder solution (PP) and polyhalite granules (PG) fertiliser treatments. Significant differences ($p < 0.05$, 2-way ANOVA followed by Tukey post-hoc test) between the treatments are indicated by different letters above the boxplot. No significant differences were seen in plants grown on different inoculum within the treatments. Each boxplot represents ten plants.

4.3.2.2 Microbial analyses

The alpha diversity of the microbial populations was measured using the Simpson index of diversity. The Simpson index of diversity (1-D) was used to ensure that the values of the Simpson index increase with increasing diversity, rather than using the original formulation of D. A lower diversity of taxa (fig. 4.3) was found in the average of the sterile inoculum treatments compared to the average of the active inoculum treatments (Kruskal Wallis, $p = 2.19 \times 10^{-7}$). In the active inoculum the no fertiliser control treatment had a lower Simpson index of diversity than all other treatments (Dunn post hoc test, all $p > 0.01$) and the polyhalite granules had a significantly lower Simpson index than the polyhalite powder (Dunn post hoc test, $p = 0.007$). As the sterile inoculum was used as a control to aid in comparison of diversity measures and microbial composition between inocula, changes between treatments in this inoculum were not be considered here.

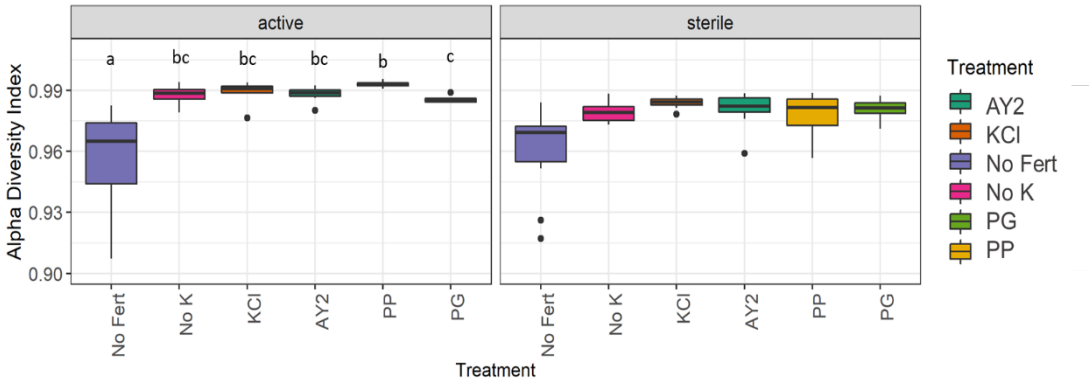


Figure 4.3 Alpha diversity indices for microbial communities of plants grown with unbalanced K fertiliser regimes. Mean Simpson index of diversity (1-D) for soil microbial communities of plants grown with no fertiliser (No Fert), no K fertiliser (No K), or different forms of K fertiliser including KCl, an adjusted yoshida (AY2), polyhalite powder (PP) and polyhalite granules (PG). Significant differences (Kruskal Wallis followed by Dunn's post-hoc test, $p < 0.05$) between the treatments are indicated by different letters above the boxplots. Boxplots represent the mean Simpson's index of diversity for the communities of 10 plants.

The effect of different K fertiliser treatments on the composition of soil microbial communities were assessed using NMDS ordination (fig. 4.4). Clear and significantly different (PERMANOVA, $R^2 = 0.33943$, $p < 0.001$) clustering was observed between the microbial communities of plants grown on the active and sterile inoculum. In addition, the no fertiliser control was separated from the main cluster of other treatments and was more dispersed than all other treatments. Further NMDS ordination plots (figs. 4.4B and C) were produced to observe the treatment positioning within the two different inoculum types. In the sterile inoculum NMDS plot the no fertiliser treatment was again more separate from the rest of the samples. Except for a few outliers, the rest of the treatment samples were clustered relatively closely, although the No K and KCl treatments were found towards the edge of this cluster, suggesting there may be some differences in microbial community in these treatments compared to the AY, PP or PG treatments. In the active inoculum, the treatments were less tightly clustered. The no fertiliser treatment behaved in a similar way to that of the sterile inoculum, being more separate from all the other treatments. However, in this plot the spacing of the KCl and No K treatments from the other K treatments is clearer. PERMANOVA of pairwise distance between microbial communities on the active and sterile inocula separately, indicated that the microbiota in all treatments were significantly different (all $p < 0.01$).

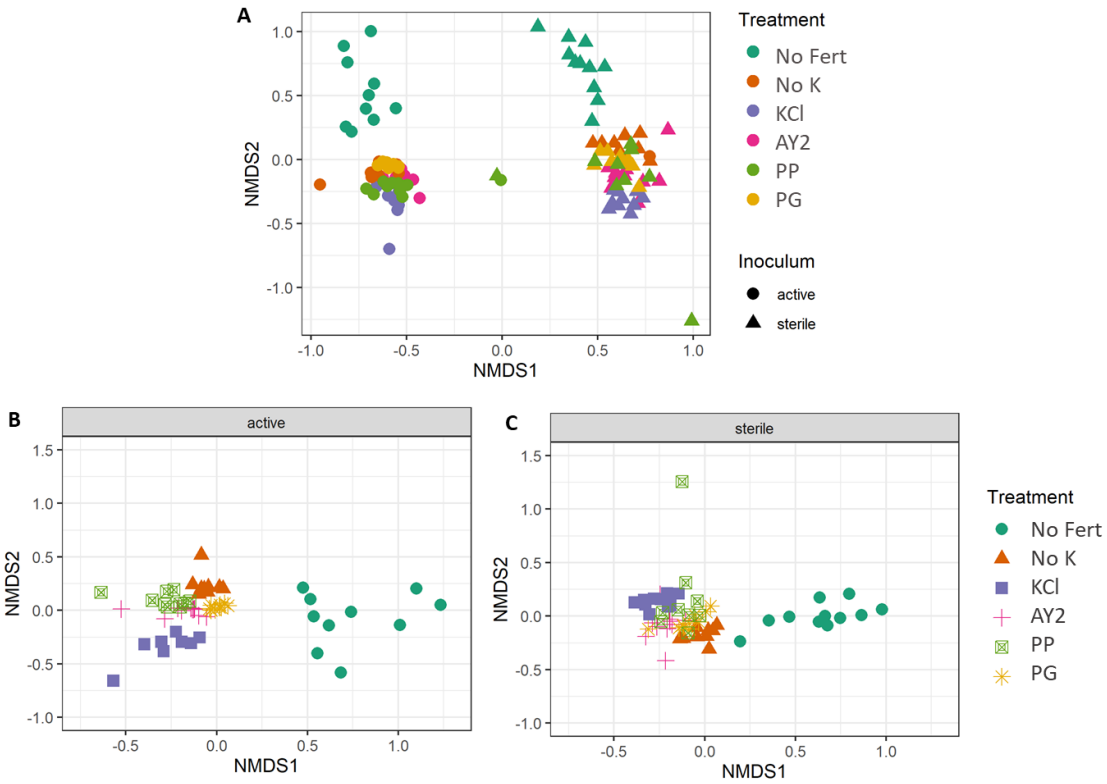


Figure 4.4 Soil bacterial community composition of plants grown with unbalanced K fertiliser regimes. NMDS plot of the bacterial community composition generated from the relative abundance Bray-Curtis matrix obtained using 16S sequencing in an unbalanced fertiliser regime using active and sterile inocula (A) and plotted individually with the sterile inoculum (B) and active inoculum (C). Each point represents the bacterial community of one pot. Different treatments included no fertiliser (No Fert), N, P and micronutrient fertilisers only (No K) , KCL, adjusted yoshida (AY2), polyhalite powder in solution (PP) and polyhalite granules (PG).

4.3.2.3 Microbial composition

Only 738 of the 4182 ASVs used for analysis originated from the active inoculum soil. The ASVs with the highest relative abundance in the original active inoculum were from the Nitrososphaeraceae, Intrasporangiaceae, Micrococcaceae, Methanobacteriaceae, Terrimicrobiaceae families. Fifty-eight taxa were found only in the original active soil and were not present in any other samples.

The 20 most abundant taxa were determined in each treatment evaluating the inocula together (fig. 4.5) and separately (appendix figs. 6.3.2 and 6.3.3) and then visualized by phylum and family to help characterise whether the distribution of different bacterial families changed between treatments. As the sterile inoculum was used as a control to aid in comparison of diversity measures and microbial composition between inocula, changes between treatments in this inoculum will not be considered here.

All treatments have a different family profile to the original soil samples. Taxa from the Planococcaceae family dominated the microbial compositions of all treatments except the original active inoculum soil. Significant differences in the relative abundance of this family were seen between the KCl and PG treatment (Kruskal Wallis, followed by Dunn's post hoc test, $p = 0.042$), No Fert and PP treatment (Kruskal Wallis, followed by Dunn's post hoc test, $p = 7.25 \times 10^{-7}$) and PG and PP treatments (Kruskal Wallis, followed by Dunn's post hoc test, $p = 0.003$). However, there were no significant differences in the relative abundance of Planococcaceae taxa between inocula types. The relative abundance of the Chitinophagaceae taxa was reduced overall in the active inoculum compared to the sterile inoculum and the No Fert treatment had a lower relative abundance of this family than all other treatments except the KCl treatment. The Intrasporangiaceae family was much higher in abundance in the active inoculum compared to the sterile inoculum and had the largest abundance in the PG treatment, however there were no significant differences between treatments in the relative abundance of this family (Kruskal Wallis, $p > 0.05$). Conversely, the Gemmataceae family had lower abundance in the active inoculum although it also had no significant differences in relative abundances between treatments (Kruskal Wallis, $p > 0.05$).

The Azospirillaceae, Burkholderiaceae, Devosiaceae and Opitutaceae families were not significantly different in relative abundance between the sterile and active inocula (Kruskal Wallis, $p > 0.05$) and the Azospirillaceae taxa and Opitutaceae taxa were also not significantly different across the active inoculum treatments (Kruskal Wallis, followed by Dunn's post-hoc test, $p > 0.05$). In both the Burkholderiaceae and Devosiaceae taxa the no fertiliser treatment had significantly lower relative abundances than the AY2 (Kruskal Wallis, followed by Dunn's post-hoc test, $p = 0.004$ and 1×10^{-4} respectively), No K (Kruskal Wallis, followed by Dunn's post-hoc test, $p = 0.021$ and 0.008 respectively) and PP treatments (Kruskal Wallis, followed by Dunn's post-hoc test, $p = 0.002$ and 0.048 respectively).

In the top 50 taxa, the Thermaceae family dominated the composition of the original sterile soil population (appendix fig. 6.3.1), however it was either not present or found at very low abundances in all other treatments. This family contains the *T. thermophilus* species which was added as a spike-in to provide a reference point for soil bacterial abundances rather than using relative abundance measurements, however the successful sequencing of the spike-in was not achieved in all samples and therefore it was not used as intended. *T. thermophilus* is not usually found in typical soil conditions as it is usually only found in geothermal environments and this uncommonness makes it a good candidate to act as a comparable sequence (Smets et al., 2016).

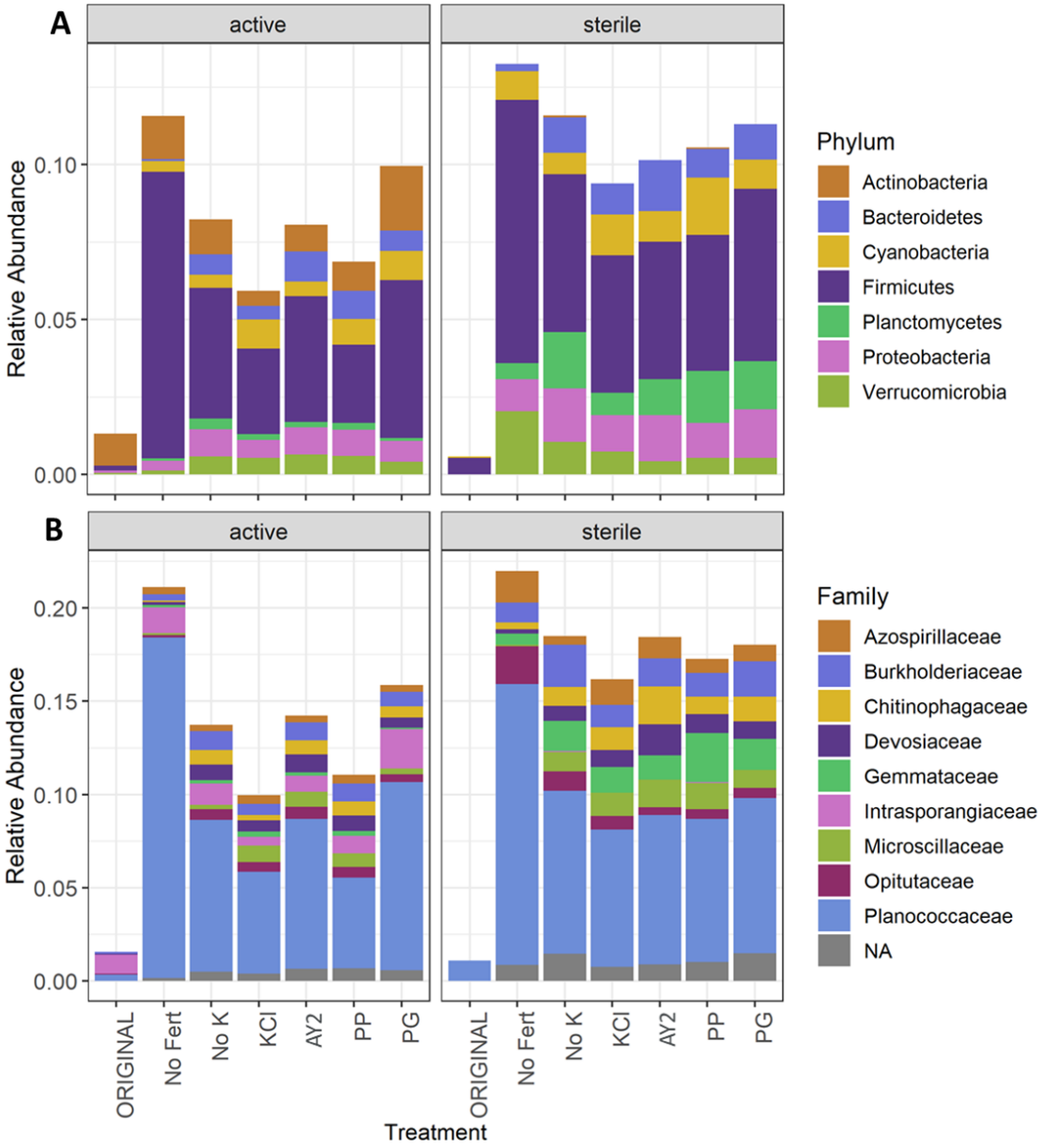


Figure 4.5 -Relative abundance of phyla and families of the top 20 taxa of soil bacterial community populations found in both inocula, from plants grown with unbalanced K fertiliser regimes. Stacked barplots of the mean relative abundance of the top 20 taxa grouped by phylum (A) and by family (B) of the soil bacterial community populations from the original inoculum (ORIGINAL) or plants grown with the following fertiliser treatments; no fertiliser (No Fert), N, P and micronutrient fertilisers only (No K), KCL, adjusted yoshida (AY2), polyhalite powder in solution (PP) and polyhalite granules (PG).

4.4 Discussion

4.4.1 Investigating the effect of balanced K fertiliser regimes on microbial communities using T-RFLPs

Soil microbial communities play an important role in soil health. A range of responses has been observed in microbial populations to fertiliser applications, however these have mostly focused on N, P or organic fertilisers. As K fertilisers are also required for crop health, it is important to assess if and how inorganic K fertilisers affect soil microbial communities and whether the multi-nutrient status of polyhalite affects these communities differently. As polyhalite is being marketed in granule form, it was also important to assess how different forms of polyhalite affected soil microbial communities. The Bray-Curtis dissimilarity (Bray & Curtis, 1957) is used to quantify the compositional differences in species populations between sites, or in this case, treatments. This beta diversity measure showed no significant differences between treatments (see fig. 4.1) whereas significant differences were seen between the sampling time points. The binding agent in polyhalite granules is composed of corn starch and this potential source of additional C in polyhalite granule treatments (PG and PGS) was expected to promote early microbial establishment. However, the absence of significant difference between these treatments suggests neither the polyhalite binding agent nor the comparison of slow-release to immediate release fertilisation methods had any effect on microbial community structure. The lack of difference between fertiliser regimes is similar to other field studies which report no significant effect of long-term chemical fertilisation on soil microbial community composition (Williams et al., 2013; Babin et al., 2019; Gu et al., 2009; Li et al., 2017; Marschner et al., 2003).

A number of previous studies have also reported changes in the rhizosphere microbial communities over the lifecycle of the plant (Chaparro et al., 2014; Edwards et al., 2018; Micallef et al., 2009; Houlden et al., 2008). Plants release root exudates that contain a variety of chemicals including sugars, amino acids, vitamins and organic acids, which can attract microbial species to the rhizosphere of the plant and act as a food source for them (Doornbos et al., 2012). Changes in the composition of root exudates throughout the plant's lifecycle may therefore be a factor in influencing the composition of rhizosphere microbial communities as seen in *Arabidopsis thaliana* (Chaparro et al., 2014) and *Avena barbata* roots (Zhalnina et al.,

2018). Whilst the changes seen in the microbial populations between timepoints as observed in this study may be due to the plant exerting an effect over the microbial communities, alternatively, it could also reflect the natural progression of development of a microbial community. As no additional microbial input was made during the experiment, the only microbes present were those already in the growing substrate or in the greenhouse environment. The lack of microbial response to fertiliser type may therefore have been due to limited bacterial diversity in the initial stages of the experiment.

The growth medium for this study was sand. Whilst this helped to reduce additional nutrient inputs, it may also have limited the opportunity for microbial communities to establish. Sand particles are generally composed of materials with a relatively inert chemical behaviour, whereas clay particles often have negative charges on their surfaces which can interact with nutrients as well as attracting bacterial cells (Mills, 2003). Hemkemeyer et al., (2018) found that bacterial taxa had different preferences for soil particle size, with the lowest bacterial abundance found in the sand fraction of the soil sampled. Bacterial biomass tends to be most concentrated in the soil fractions with smallest particle sizes e.g. silt and clay. Therefore, as well as having a limited bacterial supply, the microbial communities may also have struggled to flourish in a sand-based environment. Further experiments in soil were therefore performed using a series of unbalanced K fertiliser regimes to assess how the microbial communities responded in this scenario.

4.4.2 Investigating the effect of unbalanced K fertiliser regimes on microbial communities using 16S rRNA sequencing

The effect of different mineral K fertiliser sources on the composition of soil microbial communities was analysed using pot based experiments. The Simpson index of diversity is a measure of diversity taking into account the richness and evenness of the species (Magurran, 2004) and predicts the likelihood of two individuals from a sample being from the same species. The richness of a sample describes the number of species present within the sample, whilst the evenness is a measure of the similarity of population size of each species present. As expected, the Simpson index of diversity (fig. 4.3) showed that the soil microbial communities of plants grown using a sterile inoculum were significantly less diverse than those grown with an active inoculum, suggesting that the active inoculum may have provided a number of

additional bacterial species to those colonizing from the greenhouse, although approximately 82% of the taxa identified across the samples were not found in the original active inoculum.

Bray-Curtis dissimilarity was used to investigate the compositional differences of the bacterial populations between treatments and also showed significant differences in the bacterial populations found in the sterile and active inoculums. These differences can be observed clearly in the separation between the inoculation types in the NMDS plot (fig. 4.4). Separating out the inoculum types allowed further clarification as to how the different fertiliser treatments were affecting the microbial populations. When grouped by family, the top 20 taxa of the different treatments (fig. 4.5) emphasize this point as differences can be observed in population composition between the inoculum types as well as between treatments. There was also a large difference between the taxa composition of both the original soil used for the inocula compared to those found in the treatments. Over 7 % of the total number of taxa identified in the original active inoculum soil were not seen in any other sample. These species may not have survived the environmental changes from the field to glasshouse that they were subjected to. Collection of inoculum soils occurred in October in Yorkshire and the subsequent experiment took place in a glasshouse designed to emulate ideal rice growing conditions i.e. warm and humid. Seasonal changes such as changes to temperature (Oliverio et al., 2017; López-Mondéjar et al., 2015) and soil moisture (Castro et al., 2010) have been seen to influence soil microbial communities. Therefore, a number of factors may have altered the species composition of the treated soils compared to those from the inoculum, including plant influences and changes in temperature, soil moisture and humidity. The no fertiliser treatment only provided the plants with deionised water; therefore, this treatment provided a control showing changes in the inoculum independent of any fertiliser application

The no fertiliser treatment was significantly less diverse than all other treatments. This reduction may be linked to the reduction in plant size for this treatment as can be seen in fig. 4.2. Cereal plants can transfer up to 20 – 30 % of their photosynthates into the soil, some of which remains in the plant roots and the rest being utilized by microorganisms (Kuzyakov & Domanski, 2000). Smaller plants are likely to produce less root exudate than large plants and, as mentioned in *section 4.4.1*., plant exudates have a considerable impact on microbial

abundance and composition. However, plant size is not the only factor affecting the microbial communities as the reduction in shoot size for the plants with no K provided did not lower the Simpson index significantly (fig. 4.3).

The reduced nutrition available in the no fertiliser treatment may also have impacted the species diversity of these samples, as many microbial species, such as lithotrophs and chemotrophs, use inorganic substances as their energy source (Prescott et al., 2002). The no K treated samples were supplied with N, P and micronutrients fertilisers and the microbial diversity of these samples was higher than the treatment with no added fertiliser, however there were no significant differences in microbial diversity between the No K and K containing fertiliser treatments. These differences are similar to those seen by Ma et al. (2019) who reported significant alterations to the composition of soil bacterial communities in fields with long term N and P deficiencies but little difference when K deficiencies were present.

The slightly lower Simpson index of diversity in the PG samples compared to the PP treatment was unexpected but could be due to the additional C provided by the polyhalite granule binding agent. The release of an easily digestible C source may have provided a useful resource for the bacterial taxa colonising the soil allowing them to outcompete rarer taxa. Therefore, further work looking into the progression of the microbial communities throughout the lifecycle of the plant could help to determine whether this lowered species richness and evenness continued to later lifecycle stages.

Significant differences in the Bray-Curtis dissimilarity were seen between all treatments in both the active and sterile inoculums, although, excepting the no fertiliser treatment, all treatments were clustered more tightly in the sterile inoculum. In both inocula, the samples with no added fertiliser were more dispersed suggesting there was more variability in species composition of the populations between individual samples. The differences observed in species composition between treatments may have been due to constituent species of the inoculum soils being non-homogenized and unknown prior to application. Therefore, whilst each pot received the same quantity of inoculum, the species distribution within the applied inoculum could well have differed. Despite potential differences in species distribution within the inoculum, the more condensed clusters of all the fertiliser

containing treatments suggests that the nutrient input did play a role in the species composition. Fertiliser nutrients may impact species directly with provision of nutrients or through stimulated plant growth leading to alterations in carbon flow from the plant to the below ground system (O'donnell et al., 2001). The difference in microbial composition found between all fertiliser treatments contradicts results found in the previous section (4.3.1) where no differences were seen between treatment types. The use of soil inocula may have led to a wider range of microbial species being present in this experiment with more susceptibility to changes in fertiliser treatment.

Synthetic microbial communities utilise culturable soil microbial species to produce inocula of known composition, containing 10 to over 100 different species. These synthetic communities may provide a useful tool in future studies of fertiliser effect on microbial communities compared to using a soil derived inoculum. Microbial functional diversity refers to the ecological processes performed by a microbe and/or the ability of microbial organisms to use different substrates (Moscatelli et al., 2018). By providing a known quantity of selected bacterial strains, synthetic microbial communities may help to uncover some of the changes in microbial functional diversity that occur when using different fertiliser types as well as providing more opportunities for reproducibility. Whilst this approach still has limitations, such as lower complexity compared to natural populations and a reliance on culturable bacteria species, it may also be a useful tool in helping to understand the effect of fertiliser applications on the plant-soil interface (Vorholt et al., 2017).

The top 20 taxa identified in this study are all widely present in soils globally. The *Planococcoceae* family which is a member of the *Firmicute* phylum, dominated all the treatment types except the original inoculum soils. Therefore, it is plausible that the taxa from this family originated from the glasshouse environment, alternatively these taxa may have quickly established and outcompeted other taxa for resources. It is also possible that the predominance of this family may have been a contaminant in the samples as one of the ASVs identified as belonging to this family had a particularly high relative abundance and was found not only in the original active inoculum but also in the original sterile inoculum soil. Contamination in microbial studies is common and it is difficult to distinguish contaminating microbial DNA from that which derives from a sample. A number of precautions were taken to

reduce the number of contaminants in the data, including processing reagent only samples and negative PCR controls as well as using the *decontam* R package (Davis et al., 2018) as a software tool to address potential contaminating ASVs. Unfortunately, the reagent only samples were not successfully sequenced, however no evidence of the particular ASV was found in the PCR controls and it passed through the *decontam* software unscathed. Therefore, the data was analysed without regarding this particular ASV as a contamination, however reanalysis of the data after removing this single ASV did remove the *Planococcoceae* family from the top 20 taxa (see appendix fig. 6.3.4).

Members of the *Chitinophagaceae* family are known for their ability to digest chitin, a major component of fungal cell walls and arthropod exoskeletons. The decrease in the prevalence of this family between the active and sterile inocula, suggests that there were fewer fungal species present in these samples for these bacteria to digest. The prevalence of this family was also reduced in the no fertiliser and KCl treatments. As many fungi are involved in nutrient plant - soil interactions it is not surprising that this family was reduced in the no fertiliser treatment, however the reduction in the KCl treatment was unexpected. Therefore, further investigation of fungal species presence in KCl compared to other K fertilisers, using the Internal Transcribed Spacer (ITS) region of DNA, could be informative, although this future work would be better suited to another crop species as the soil of rice grown in flooded fields is dominated by bacteria (Ding et al., 2019).

It can clearly be seen that the Thermaceae family dominates the composition of the sterile inoculum. This represents the *Thermus thermophilus* spike-ins that were added to each sample to provide more accurate abundance data for the samples. Whilst the spike-ins were successfully amplified and sequenced, they were not found to be present in many of the other samples and if so at very low read numbers. This suggests that the concentration of the spike-in added to the samples was too low as well as showing that the sterilisation of inoculum soil did successfully reduce the overall abundance of species present in this inoculum.

In this study the top 20 taxa found in the treatments across both inocula were analysed, showing that across the treatments there were many similarities in the relative abundance of the most prevalent taxa. Further analysis found that splitting the inocula

categories and examining the top 20 taxa changed some of the species present (see appendix figs. 6.3.2 and 6.3.3). However, in general the relative abundances of the taxa were still relatively similar across the treatments, with more differences seen between inocula than between treatment types.

Whilst it is useful to know the most prevalent taxa within a sample, this information is not necessarily very informative of soil microbiome functional diversity. In general, soil bacterial communities are dominated by a small number of species, whilst their richness is associated with rare species with unknown ecological roles. It is estimated that only 0.4% of bacterial species are considered common with the remainder of the species being endemic with low relative abundances (Bickel & Or, 2021). Furthermore, those species which are common across soils are usually successful across a variety of ecological and environmental conditions and as such are often poor indicators of changes in environmental conditions or community compositions (Bickel & Or, 2021). Unfortunately the rare species which are considered to be ecologically important are often those which are poorly characterised with many phyla virtually unstudied (Janssen, 2006). Another factor worth considering is that the taxa of all treatments, regardless of the inocula used, have a relatively similar profile of most prevalent taxa, suggesting that these may have already been present in the greenhouse environment.

Species identification is useful to identify differences between treatments, however the functionality of the species within the microbiome may not be vastly altered. There is evidence to suggest that whilst microbial ecosystems may differ in their taxonomic composition, the functional categories of microbes present are often similar (Taxis et al., 2015) suggesting that taxonomy may mask the functionality of the microbial population. Therefore the use of assays investigating soil microbial substrate utilisation (Konopka et al., 1998) or enzyme activities (Waldrop et al., 2000) may be useful in future studies to explore whether different K fertilisers alter the functions performed by the soil microbiome.

4.4.3 Overall discussion and conclusions

Potassium fertiliser type had no significant difference on microbial populations when plants were grown in sand, nor on richness and evenness measures when plants were grown in

soil, whereas microbial species composition was found to differ between treatments in soil grown plants. Whilst bacterial species were seen in the soil of the sterile inoculum this population likely represented the population of the glasshouse microbiome. The significant difference shown between the active and sterile inocula species composition suggests that the active inoculum did provide additional bacterial species. Furthermore, the use of a soil inoculum did not have an effect on shoot growth as no significant differences in shoot growth were observed between the sterile and active inocula.

The use of polyhalite and other K fertiliser sources did not negatively affect the soil microbial communities with the only change in diversity being found between the two polyhalite treatments. Furthermore, across the K fertiliser containing treatments, similar relative abundances of the twenty species with the highest abundance was found.

The changes in microbial species composition may be due to nutritional differences between the fertiliser regimes, differences in nutrient release profile or changes to plant exudate composition due to nutrient differences in the fertiliser regimes. However, there may also have been differences in the species which colonised the pots from the greenhouse environment or in the initial species composition of the inoculum added to each pot when used. In addition, pot experiments have limitations as they inherently have a reduced microbial diversity or altered community structures compared to field studies as there is a limited pool of available microorganisms in the environment. Therefore these experiments are often not indicative of how a change in variable might affect microbial communities in a field setting (Berg et al., 2016). As these experiments gained information from individual plant root systems, they missed the interactions that occur between plants in a field setting as well as the surrounding soil systems. Therefore, future work looking at the impact of different K fertiliser sources on microbial populations in field settings would be informative. The use of a synthetic microbial community as an inoculum for greenhouse-based studies would also be beneficial, allowing further investigation into species and functional diversity changes.

Chapter 5 – Overall Conclusions and Perspectives

5.1 Final Conclusions

The use of polyhalite as a K fertiliser has been studied to a limited extent, with most literature focussing on field trials directly comparing K fertiliser sources without providing a balanced nutritional regime to complement them. While some previous studies suggest that polyhalite may promote plant growth compared to other K fertilisers (Pavuluri et al., 2017; da Costa Mello et al., 2018b,a; Zhou et al., 2019; da Costa Mello et al., 2020; Lillywhite et al., 2020) the results of this study do not support this conclusion. The main conclusions of this study are:

1) Polyhalite does not affect rice growth traits in balanced fertiliser regimes. In unbalanced fertiliser regimes, all rice growth traits are unaffected by K fertiliser type except thousand grain weight which was significantly higher in KCl treated plants than polyhalite treated plants. Different rice cultivars also had no difference in rice growth traits when supplied with different K fertiliser types in balanced fertiliser regimes.
2) Nutrient composition of plants provided with different K fertilisers is variable with no consistent change in nutrient content between treatments.
3) T-RFLP analysis of microbial community composition between different balanced K fertiliser treatments found no differences in community structure between treatments. 16S rRNA analysis found differences in the richness and evenness of the microbial community between the 'no added fertiliser' treatment and all other treatments, and also between the' polyhalite powder' and 'polyhalite granules' treatment. Furthermore, the species composition was significantly different between all treatments.

Crop yield is the one of the most important agricultural traits and therefore the performance of polyhalite as a K fertiliser is linked to yield production. The efficacy of polyhalite as a potassium fertiliser for rice plants was comparable to that of KCl and K_2SO_4 with no differences in root, shoot or panicle growth traits found in this study when nutrients were fully balanced or when K fertilisers were directly compared. As polyhalite can be used as a slow release fertiliser it may provide additional on-farm benefits including reducing labour costs due to fewer fertiliser applications being required. Fewer fertiliser applications also provide farmers with more time, an important commodity in any business, as well as the potential to reduce farm emissions due to decreased machinery usage. Reduced machinery use may also

improve soil conditions as the weight of farm machinery can lead to soil compaction which can hamper crop growth.

Nutrient responses to polyhalite were inconsistent across the different experiments within this study, regardless of whether investigating balanced or unbalanced fertiliser regimes, with no single nutrient retaining a consistent increase or decrease in concentration in the polyhalite treatment compared to other treatments used. Whilst nutrient responses may have been variable, it is clear from figure 5.1 that in the majority of cases there was no significant difference in nutrient content between treatments. Across the different experiments performed in this thesis, the majority of differences in nutrient content were found at the vegetative sampling stage, with ten significant differences between treatments found at the vegetative stage whereas later sampling stages had far fewer significant differences in nutrient content between treatments, with both the flowering and grain sampling stages only having five significant differences between treatments across all the nutrients measured. Other polyhalite studies in tomato (da Costa Mello et al., 2018b, 2020), tea (Zhou et al., 2019), maize (Dal Molin et al., 2020; Lillywhite et al., 2020) and barley (Lillywhite et al., 2020) have only focussed on plant nutritional content at the harvesting stage, however observing nutrient content through the lifecycle of the plant was important to establish whether nutrient changes at earlier lifecycle stages influenced plant morphology or yield measurements. The general lack of nutritional differences between treatments suggests that polyhalite performs as well as other K fertilisers and therefore factors such as its slow-release chemistry and cost analysis should be analysed to give an indicator of other potential advantages or disadvantages that polyhalite may have in a farm setting.

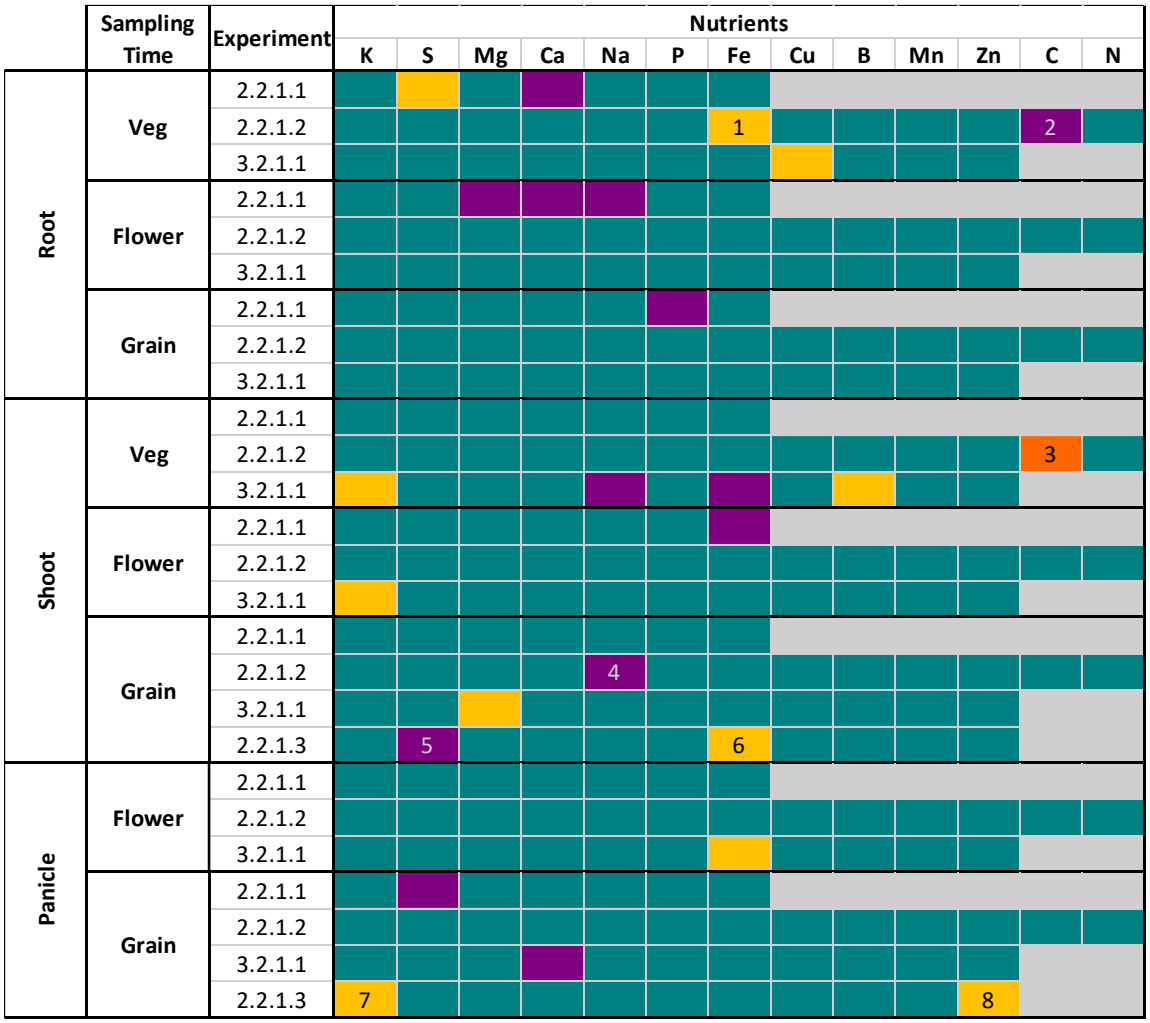


Figure 5.1 – Changes in nutrient content between polyhalite and other K treatments across all experiments. Significant increases of a nutrient content in polyhalite treated plants compared to other treatments is marked in purple and significant decreases in a nutrient content in the polyhalite treated plants compared to other treatments are marked in yellow. Significant differences in nutrient contents of plants with two different polyhalite treatments are marked in orange. Numbered boxes demonstrate the specific treatment differences where significant differences were found and multiple treatments were used.

The composition of microbial communities can affect crop growth and yield, both positively due to their roles in mobilisation of soil nutrients, suppression of phytopathogens and inducing plant defensive responses and negatively by utilising plant available nutrients and acting as plant pathogens. Analysis of the soil microbial communities using T-RFLPs found no significant differences in microbial community composition between a number of balanced K fertilisers, however investigation of unbalanced fertiliser regimes using Illumina sequencing found differences in bacterial species composition although little change was observed in the total number or distribution of species of the bacterial populations. Whilst the K, N, P and micronutrient application rates were the same in the unbalanced fertiliser treatments, the S, Ca and Mg nutrient additions were not balanced. This suggests that these nutrients may have been affecting the microbial community composition, however it is not possible from the experiment in this study to establish how or which of these nutrients were having the most effect.

5.2 Problems and Improvements to Experimental Design

For all experiments the control treatment was an adjusted yoshida (AY1 or AY2) nutrient solution made up from chemical salts to reflect the nutrient profile of polyhalite. Yoshida medium (Yoshida et al., 1976) is used as a nutrient solution for rice plants and is considered to contain quantities of each nutrient that are optimal for rice growth. By adjusting the yoshida medium to match the polyhalite nutrient profile, plants may not always have had the optimal nutrition for growth during their lifecycle. An alternative approach may have involved added nutrients to the polyhalite treatments to match them to the Yoshida medium, thereby maintaining the optimal nutrient profile for plant growth. Furthermore, Yoshida medium is primarily used in hydroponics with the nutrient solution being replenished weekly, therefore using this system in pots and only applying the fertiliser 3-4 times at the beginning of the plants' growth may have provided the plants with not only non-optimal nutrient distribution but also a lower application rate than is ideal. Visually it was apparent that plants had low N content, as their leaves were frequently a light green colour at the flowering and grain sampling stages. As plants can often store excess nutrients within their vacuole and in structural components, it was necessary to maintain low levels of fertilisation to allow for comparison of fertilisers without an excess of nutrients being able to build up within the plant

and reduce the comparative effects. Matching the adjusted yoshida to the polyhalite nutrient profile also allowed easy comparison of the balanced and unbalanced fertiliser regime experiments.

Changes in harvest timings between the experiment in hydroponics compared to all other experiments may help to explain some of the inconsistencies in nutrient content differences. For the hydroponics data, at each sampling stage all plants were harvested on the same day. Due to concerns regarding developmental stages of the plant affecting nutrient distribution, harvesting timepoints in later experiments coincided with specific developmental stages in order to reduce these potential effects. In the cultivar experiment, the growth of two plants per pot made it difficult to harvest both plants at the same developmental stage because, despite having the same genotype, they frequently differed in their flowering times.

Another variable which changed between the experiments was the growth substrate, with hydroponics, sand and soil being used. The changes in growth substrate were important for a comprehensive evaluation of plant responses to polyhalite, but also in the case of the unbalanced fertiliser experiment, to provide some additional nutrition to allow for adequate growth of plants when not provided with a full complement of essential nutrients in the fertiliser application. Nutrient recovery is influenced by soil type and also by the presence and availability of nutrients within the soil matrix. For example, S deficiencies most frequently occur in sandy soils while Ca and Mg deficiencies are more common on acidic sandy soils (Hillel, 2008). Differences in soil nutrition can be due to a number of factors including particle size, organic matter content and the mineral source of the nutrients. As all the fertiliser treatments were applied prior to the vegetative sampling, excess nutrients could either be retained in the soil matrix or in the leachate, which remained available for the plant to use throughout the course of the experiment. Fine to medium textured soils have a larger surface area and hence better retention of nutrients and water compared to coarse, sandy soils (White, 2009). More nutrients may therefore have been retained in the experiments using soil compared to those using sand. A noticeable difference was also observed in root growth phenotype between the growth substrates: in sand the roots were much thicker and had fewer root hairs compared to roots grown in soil, which may have reduced their capacity to take up nutrients. Unfortunately, the difficulty of disentangling roots from soil made it

impossible to measure their dry weight and so it was not possible to make a quantitative comparison of these phenotypes for the different growth substrates.

Polyhalite contains K, S, Mg and Ca and therefore when directly comparing fertilisers it was hypothesised that plants treated with polyhalite would have higher Mg and Ca contents than K_2SO_4 and KCl treatments and higher S content than the KCl treatment. Whilst the S content was higher in the polyhalite treated plants than KCl treated plants, neither the Mg or Ca contents changed between treatments which may have been due to an ample supply in the original soil. Therefore, measurement of the pre-existing nutrient content of soil used with the unbalanced fertiliser regimes would have been beneficial to help identify whether the soil already contained suitable quantities of the different nutrients required by plants. Additionally, as soil pH can alter nutrient availability for plants this would also have been a useful soil measurement across all the experiments.

The use of a soil based microbial inoculum allowed for comparison of K fertiliser effects on a larger and more field realistic population of microbial species. Ideally the soil inoculum would have been collected from a field with a rice crop to provide an even more realistic starting point for the experiment. However, this experiment fell within COVID-19 restrictions periods in the UK, where the importation of soil from a rice growing region would have been very challenging, therefore the pseudo-paddy field soil collected from the University of York campus was used as an alternative, if less realistic solution.

5.3 Future Work

The only difference seen in yield traits was a reduced TGW in the KCl treatment compared to the polyhalite treatment when K fertilisers were directly compared. It is likely that the lower TGW in the KCl treatment was related to the absence of applied S in this treatment which was also found to significantly reduce shoot and panicle S content in KCl treated plants. Reduced S content could be detrimental in crop species which have higher S requirements, for example members of the *Brassicaceae* family (Hawkesford & De Kok, 2006) which may mean polyhalite is a preferable K source for these crops. Investigating the use of polyhalite in crop species with a higher S requirement would be beneficial for understanding its performance in these crops compared to alternative K fertilisers. This is of increasing

importance due to the increase in soil S deficiencies worldwide (Feinberg et al., 2021) which may lead to a higher necessity for S fertilisation for crop growth, and therefore using polyhalite as a multi-nutrient fertiliser could be advantageous compared to KCl.

As well as affecting crop yields, TGW can also impact quality traits (Bhattacharya, 2011), therefore the inclusion of rice quality traits, such as grain dimensions, grain chalkiness, milling properties and cooking qualities in future studies could be valuable.

No differences were seen in crop growth attributes when comparing slow and fast releasing forms of polyhalite or when comparing different K sources in an unbalanced fertiliser regime. As well as potentially reducing the number of fertiliser applications required and allowing improved flexibility for fertiliser applications, the slow release chemistry of polyhalite may reduce nutrient leaching in the field. Nutrient losses through leaching did not occur in this study, as all leachates were captured to allow the plants continued access to these resources. Therefore, laboratory studies using plants grown in leachate columns could provide an insight into plant access to soil nutrients combined with nutrient movement through the soil.

Differences in fertiliser price as well as reduced labour costs due to decreased frequency of fertilisation applications can influence the economic gain of choosing one fertiliser compared to another. Economic analysis comparing the use of polyhalite and K_2SO_4 for tea growth found that polyhalite provided an economic benefit in the last two years of the study with an increased revenue of US \$243 ha^{-1} when using polyhalite in the penultimate year of the study despite no significant difference in tea yield between polyhalite and K_2SO_4 treated crops in this year. In the final year of the study the yield was significantly higher than in polyhalite treated crops than those grown with K_2SO_4 and provided an increased revenue of US \$1982 ha^{-1} (Zhou et al., 2019). In contrast to rice, tea is a perennial crop which can by profitable for up to 100 years (Zhang et al., 2020) and therefore the increased revenue found for tea in the third year of the study may not be seen in rice production, especially as in the first year of the study by Zhou et al (2019), the polyhalite treated tea had a decreased revenue compared to the K_2SO_4 treated crops, despite a similar yield. As polyhalite does not affect rice yield it may be prudent for future studies to consider perform cost analysis to establish any alternative economic benefits it may provide in rice.

Iron had the highest number of significant differences between treatments across the different experiments performed with 5 changes overall. Two of these changes were increased shoot Fe content, occurring at the vegetative or flowering timepoints in two different experiments. There were also three occurrences where polyhalite had decreased Fe content compared to other treatments, however there was no consistency in the plant part of sampling timing for any of these decreases. The quantity of Fe within polyhalite is too low to have any impact on plant growth or nutrient content, however the changes seen in plant Fe content across the experiments suggest that polyhalite may be influencing Fe uptake or distribution. As the results from this experiment are inconclusive in this regard, further work investigating how polyhalite might affect plant Fe content may be beneficial. This work would be particularly prudent in acidic lowland rice fields where Fe toxicity, due to excessive Fe^{2+} within the soil, is one of the most important constraints to rice production (Becker & Asch, 2005).

Soil salinity is another yield limiting factor in global rice production. Rice plants have increased sensitivity to salinity at early lifecycle stages and saline conditions can delay flowering and decrease grain yield (Zeng et al., 2001). As well as concerns raised over the use of KCl as a K fertiliser in saline conditions due to its Cl content (Farooq et al., 2018), increased Na uptake is a factor in plant response to salinity (Lee et al., 2003). Whilst plant Na content was largely not significantly different between treatments in this study, there were three occurrences where polyhalite treated plants had higher Na content, two of which were at early lifecycle stages. This study also found that low concentrations on Cl had no significant affect in rice plant growth. Therefore, future investigations could consider the impact of different K fertilisers in saline conditions to help determine how polyhalite performed as a K fertiliser in these conditions.

Changes in plant exudate composition throughout the lifecycle of the plant is one of the methods plants use to influence the composition of the rhizosphere microbiota (Shi et al., 2011). Reduced root size has been shown to decrease root exudate production (Přikryl & Vančura, 1980). Whilst root dry weights were not measured in the 16S rRNA microbial experiment, the shoot dry weights of plants grown without fertiliser additions were far smaller than plants grown with fertiliser applications and visual observations confirmed that these

plants had smaller roots. Additionally, the richness and evenness of these microbial communities of plants grown without any fertiliser application was significantly different to that of the other treatments. To untangle the plant size effect on root exudates from the fertiliser effects on microbial community, future studies could collect root exudates from plants grown with different fertiliser treatments and apply these to soils at a uniform concentration to help identify whether difference in exudate quantity was one of the influencing factors driving differences in microbial richness and evenness.

As aforementioned the soil inoculum used in microbial experiments was not the most realistic for rice growth therefore future work may benefit from the use of either soil from rice fields as an inoculum or synthetic microbial communities with known functions, which could contribute to our understanding of how fertilisers influence these populations and further illuminate any impact of inorganic chemicals on plant-soil interactions. These synthetic communities provide the added advantage of allowing reproducibility, providing researchers with more flexibility in altering variables (Vorholt et al., 2017).

The top 20 taxa identified were relatively consistent between the treatments, suggesting that the main differences found between species composition were found in taxa with a lower relative abundance. The most prevalent taxa within the soil are not necessarily informative regarding the functionality of the species within a microbial community and a limitation of this study lies in not investigating the effect of K fertilisers on the functional diversity of soil microbial communities. Functional diversity of soil microbial communities can be evaluated, for example, by recording changes in cellular metabolism of a variety of substrates including; sugars, proteins, amino acids and fatty acids. Such approaches used in combination with 16S rRNA sequencing, could provide a broader picture of the changes in composition and functionality occurring in the soil microbial communities when faced with different K fertiliser sources.

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6. Appendix

6.1 Supplementary Figures for Chapter 2

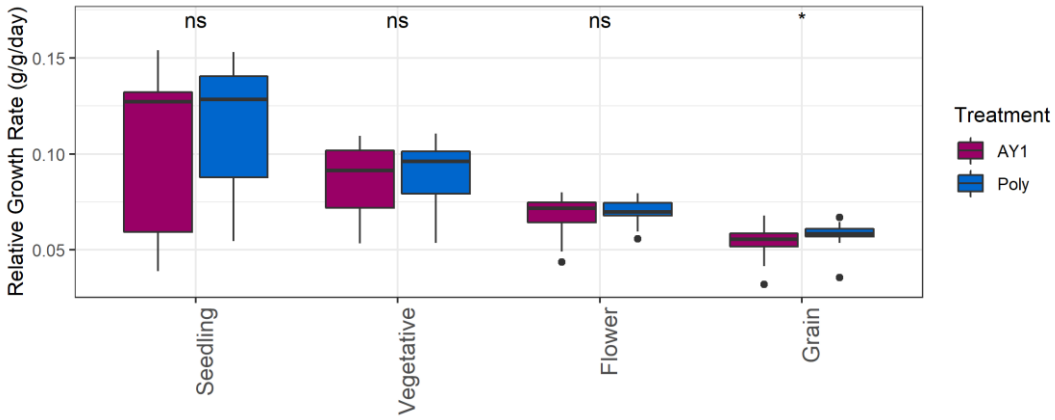

Figure 6.1.1 – Relative growth rate of plants at the seedling, vegetative, flowering and grain stages of plants grown hydroponically with a balanced K regime. Boxplots of relative growth rate of plants grown in hydroponics with an AY1 or polyhalite nutrient solution and sampled at four different lifecycle stages. Significant differences (Mann Whitney U test $p < 0.05$) between treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$).

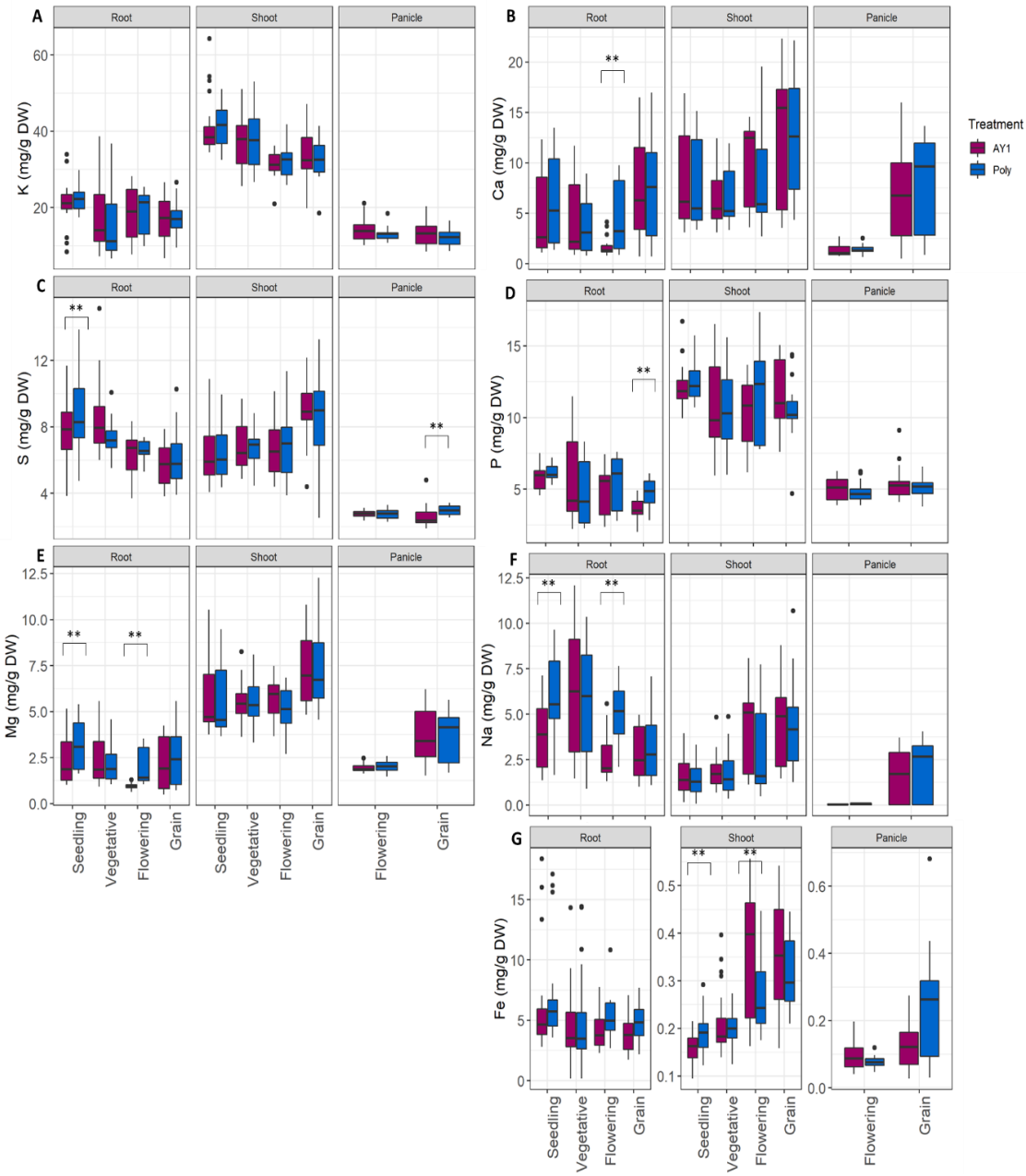

Figure 6.1.2 – Root, shoot and panicle nutrient content at the seedling, vegetative, flowering and grain stages of plants grown hydroponically with a balanced K regime. Root, shoot and panicle panels of boxplots of K (A), Ca (B), S (C), P (D), Mg (E), Na (F) and Fe (G) content. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$ Student's T-test or Mann-Whitney U test) between treatments are indicated by asterisks (* $p < 0.05$ ** $p < 0.01$ *** $P < 0.001$) above boxplots. Each boxplot represents thirty plants.

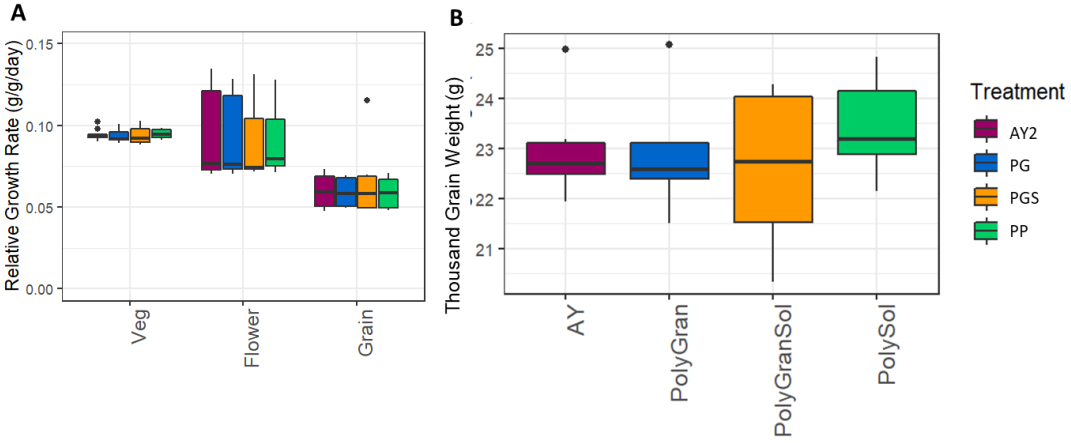

Figure 6.1.3 – Relative growth rate and thousand grain weight of rice plants grown in sand with a balanced K regime. Boxplots of relative growth rate (A) of plants grown in hydroponics with an AY1 or polyhalite nutrient solution and sampled at three different lifecycle stages and thousand grain weight of plants (B). No significant differences were seen between treatments (ANOVA, $p < 0.05$).

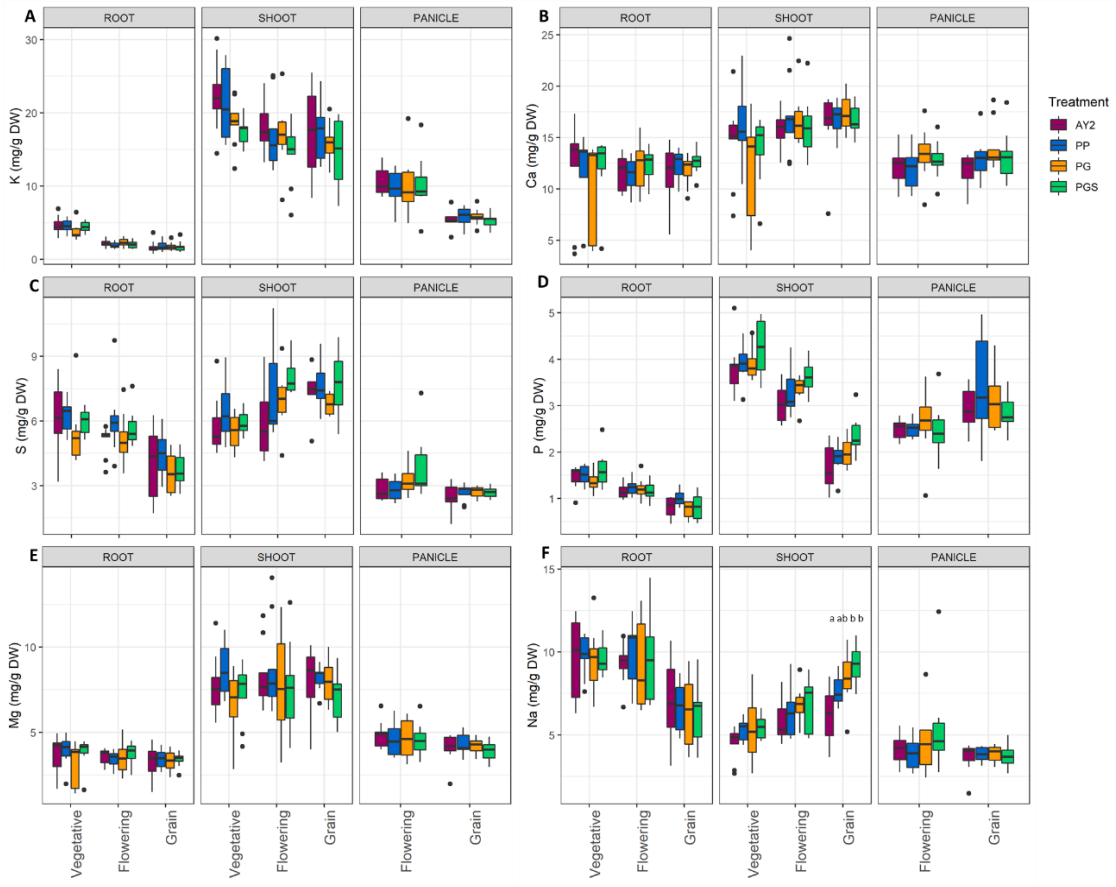

Figure 6.1.4 – Root, shoot and panicle nutrient content at the vegetative, flowering and grain stages of rice plants grown in sand with a balanced K regime. Root, shoot and panicle panels of boxplots of K (A), Ca (B), S (C), P (D), Mg (E) and Na (F) content. See fig.2.1. caption for explanation of boxplot. Plants were grown with an adjusted yoshida (AY2), a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG) and sampled at three different lifecycle stages. Significant differences ($(p < 0.05)$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are indicated by different letters above the boxplots. Each boxplot represents nine plants.

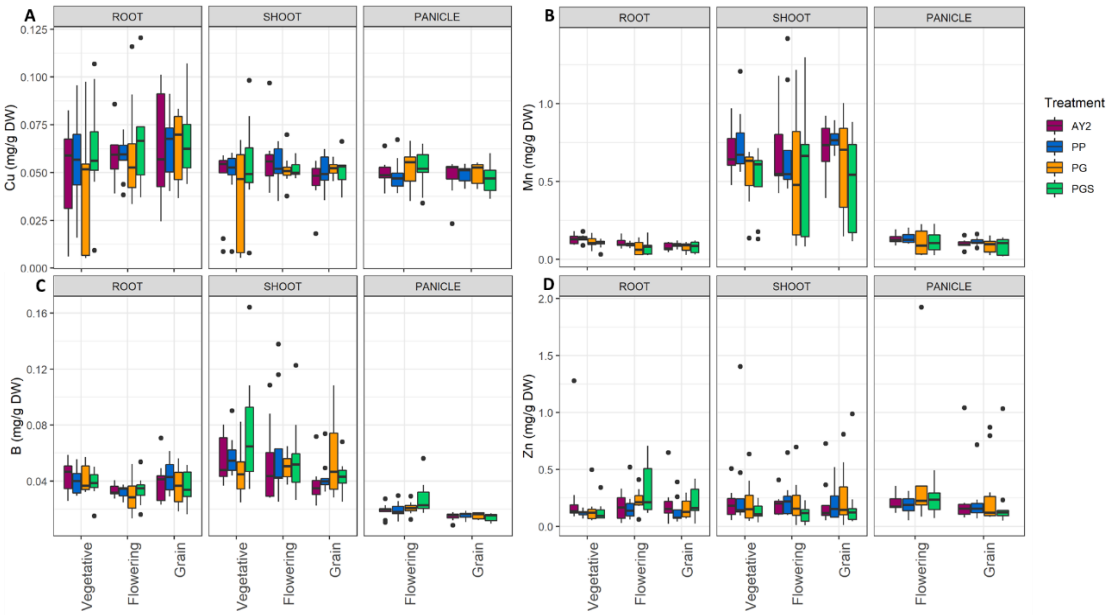

Figure 6.1.5 – Root, shoot and panicle nutrient content at the vegetative, flowering and grain stages of rice plants grown in sand with a balanced K regime. Root, shoot and panicle panels of boxplots of Cu (A), Mn (B), B (C) and Zn (D) content. See fig.2.1. caption for explanation of boxplot. Plants were grown with an adjusted yoshida (AY2), a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG) and sampled at three different lifecycle stages. No significant differences were observed (($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments. Each boxplot represents nine plants.

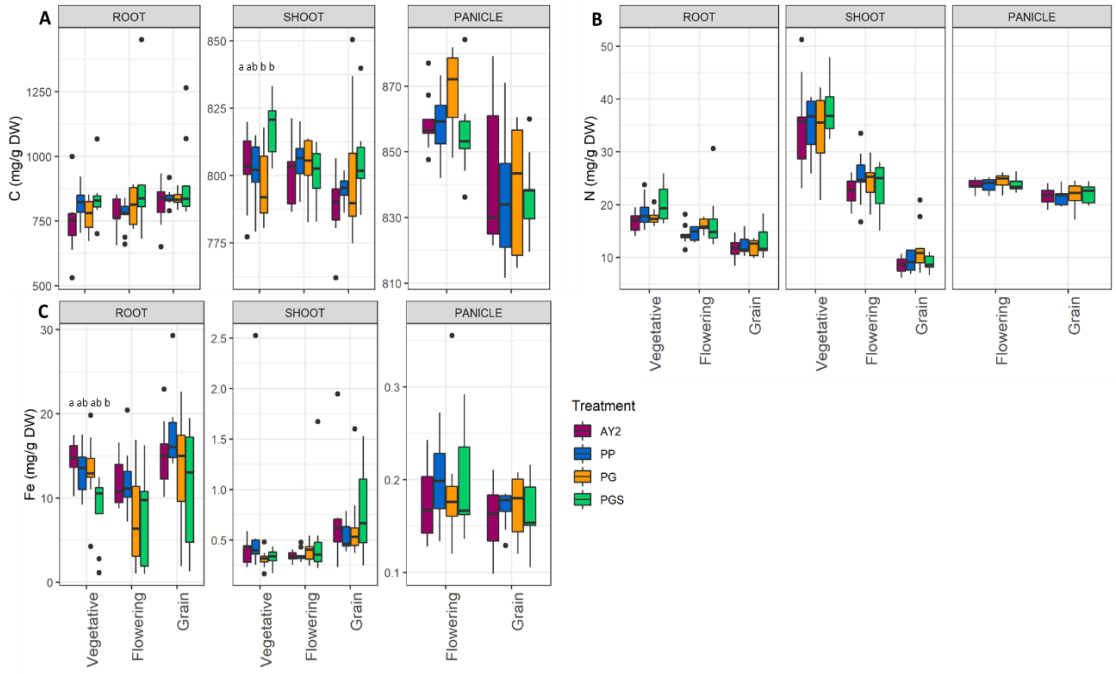

Figure 6.1.6 – Root, shoot and panicle nutrient content at the vegetative, flowering and grain stages of rice plants grown in sand with a balanced K regime. Root, shoot and panicle panels of boxplots of C (A), N (B) and Fe (C) content. See fig.2.1. caption for explanation of boxplot. Plants were grown with an adjusted yoshida (AY2), a polyhalite powder solution (PP), polyhalite granules solution (PGS) or polyhalite granules sprinkled on the top of the pot with a NP + micronutrients solution added (PG) and sampled at three different lifecycle stages. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are noted by different letters above boxplots. Each boxplot represents nine plants.

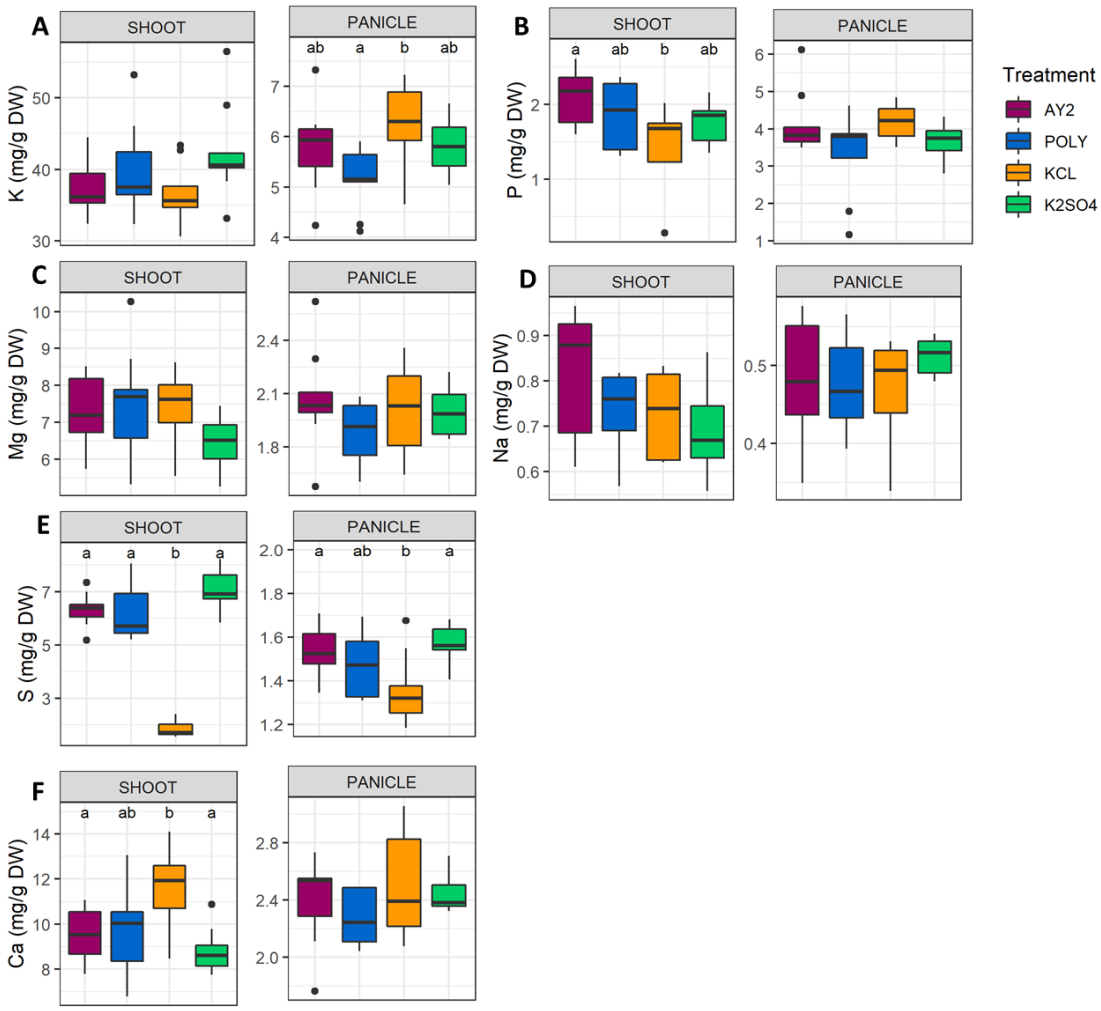

Figure 6.1.7 – Shoot and panicle macronutrient content at the grain stages of rice plants grown in soil with an unbalanced K regime. Shoot and panicle panels of boxplots of K (A), P (B), Mg (C) Na (D), S (E), and Ca (F) content. See fig.2.1. caption for explanation of boxplot. Plants were grown in pots with an AY2 solution, KCl granules, K_2SO_4 granules or polyhalite granules (Poly). Significant differences ($(p < 0.05)$ ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are noted with different letters above the boxplots. Each boxplot represents twelve plants.

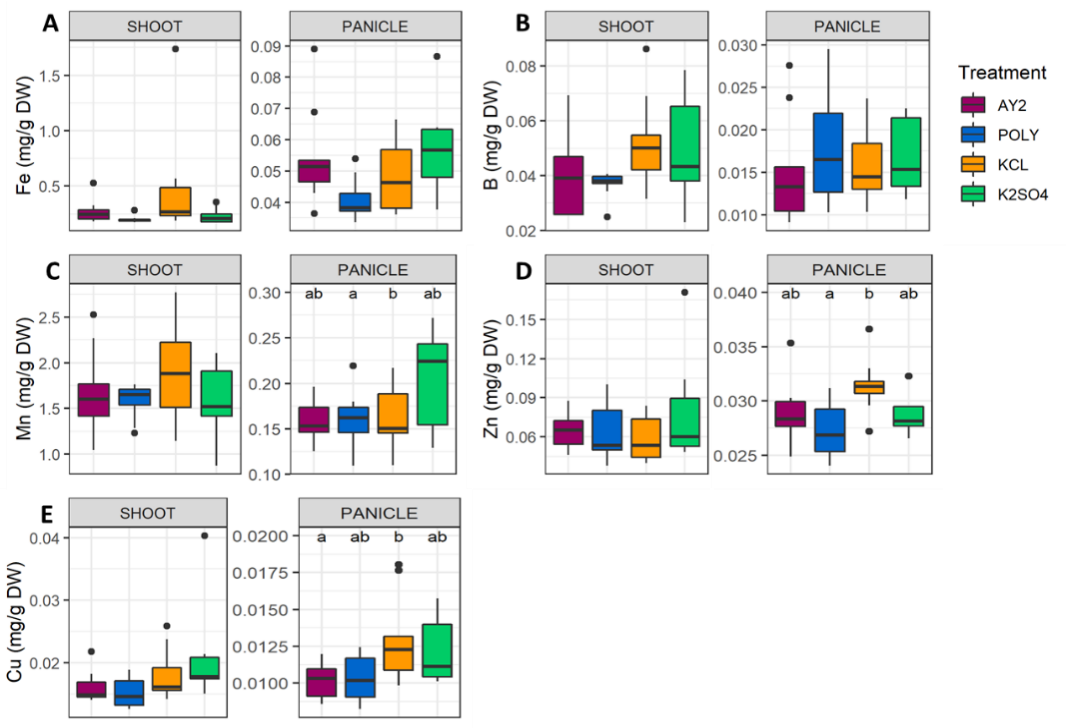

Figure 6.1.8 – Shoot and panicle micronutrient content at the grain stages of rice plants grown in soil with an unbalanced K regime. Shoot and panicle panels of boxplots of Fe (A), B (B), Mn (C) Zn (D) and Cu (E) content. See fig.2.1. caption for explanation of boxplot. Plants were grown in pots with an AY2 solution, KCl granules, K_2SO_4 granules or polyhalite granules (Poly). Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments are noted with different letters above the boxplots. Each boxplot represents twelve plants.

6.2 Supplementary figures for Chapter 3

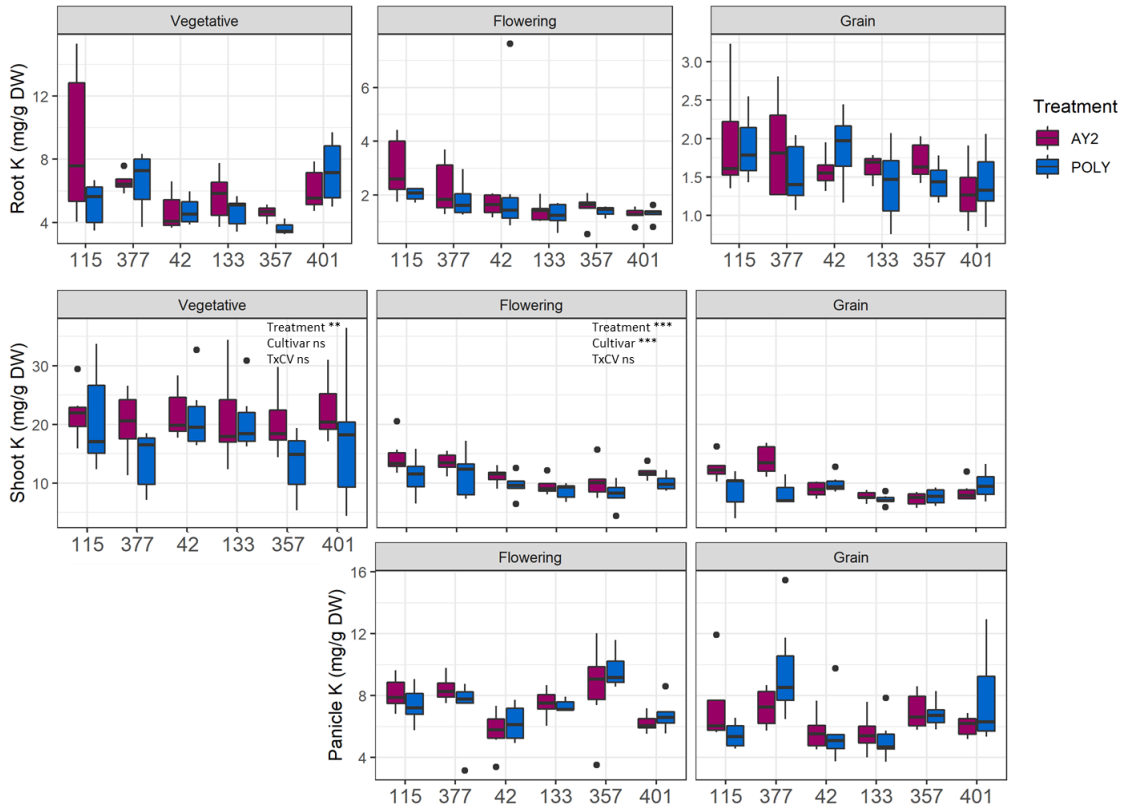

Figure 6.2.1 – Root, shoot and panicle K content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

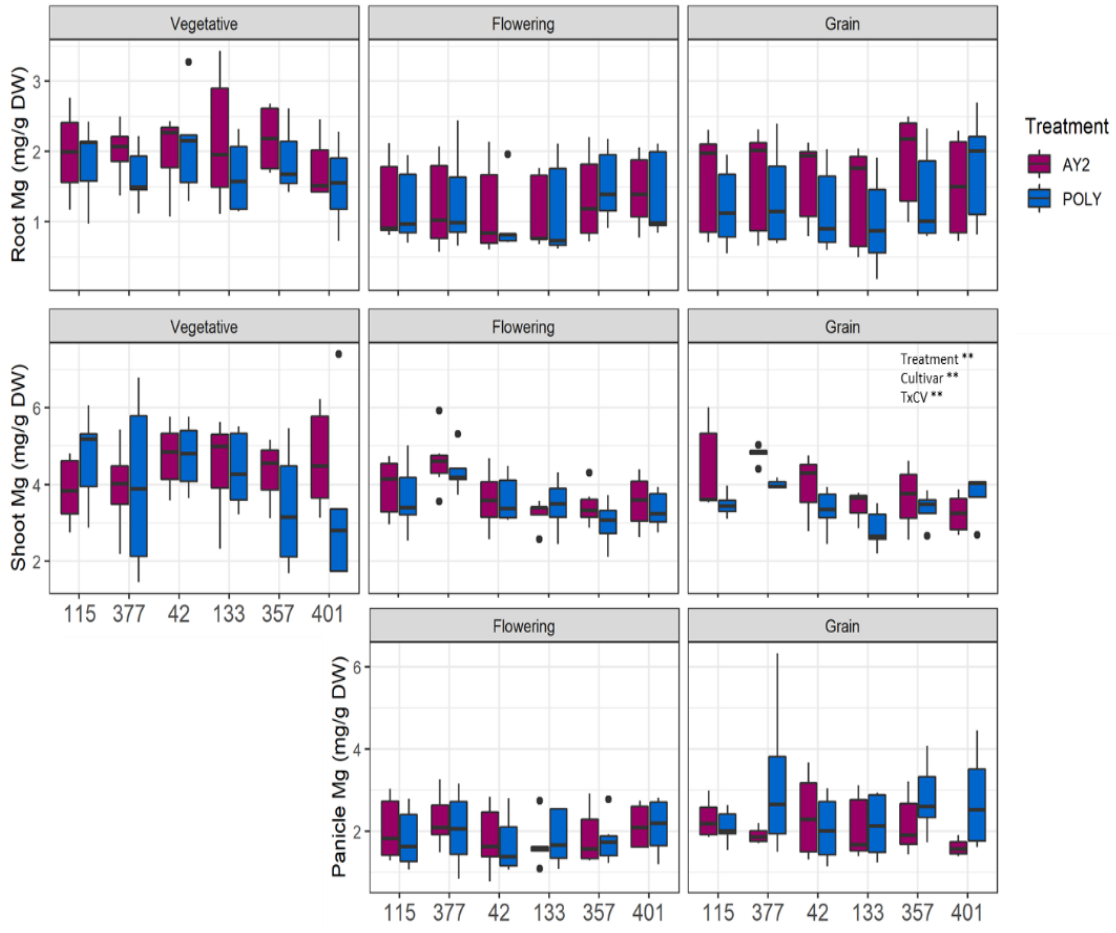

Figure 6.2.2 – Root, shoot and panicle Mg content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

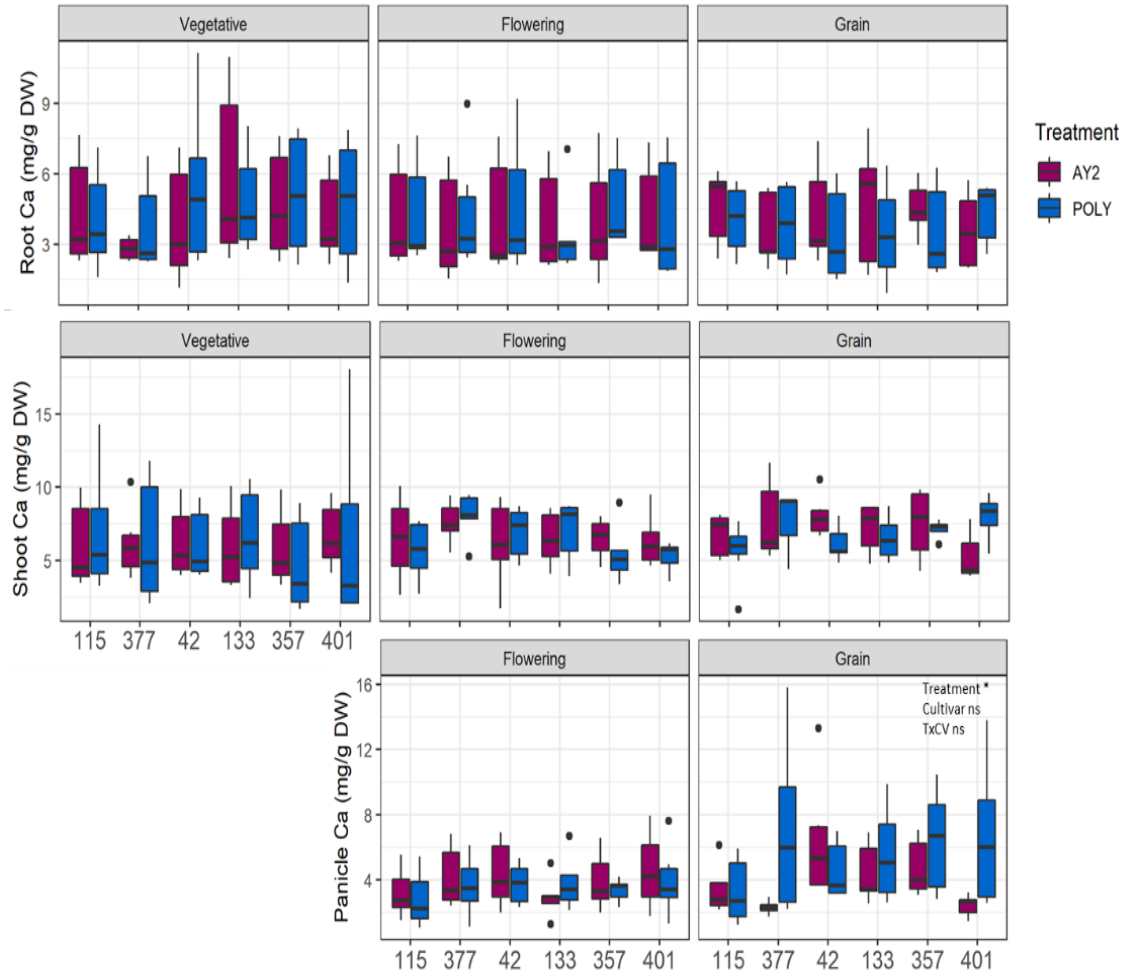

Figure 6.2.3 – Root, shoot and panicle Ca content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences (($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

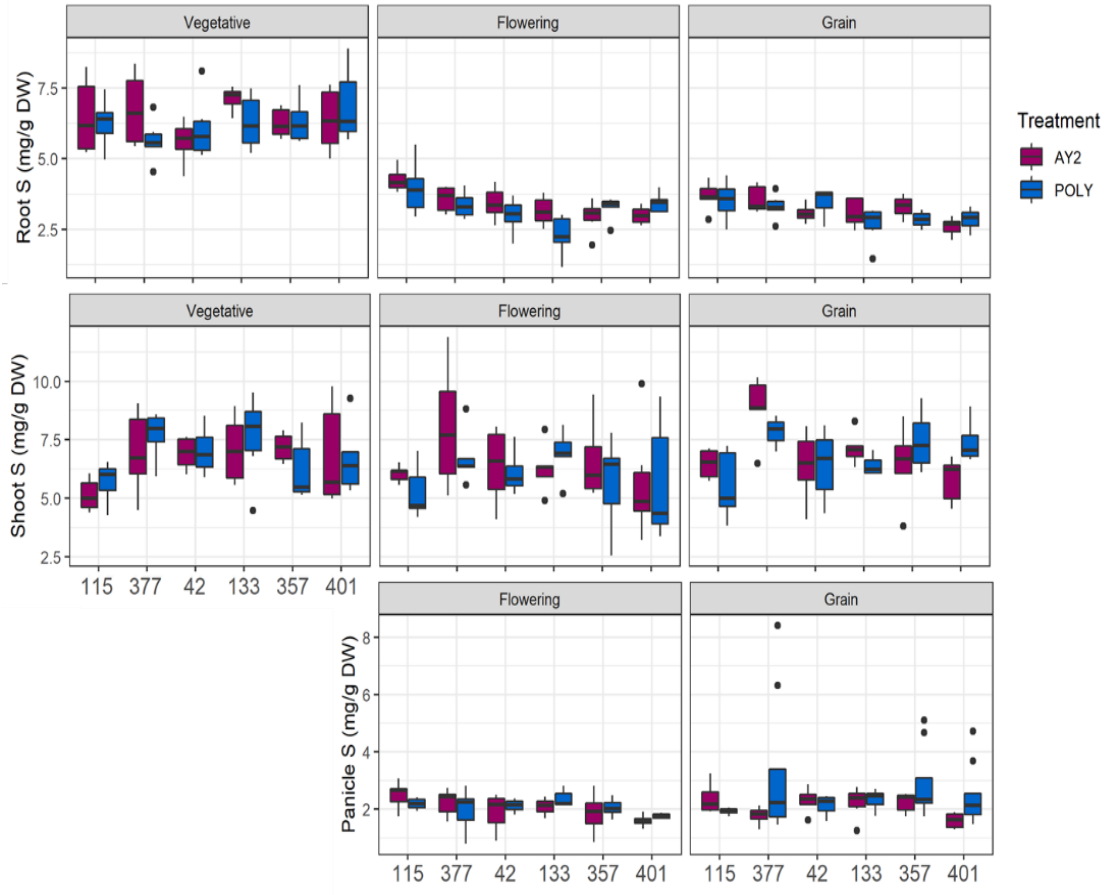

Figure 6.2.4 – Root, shoot and panicle S content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

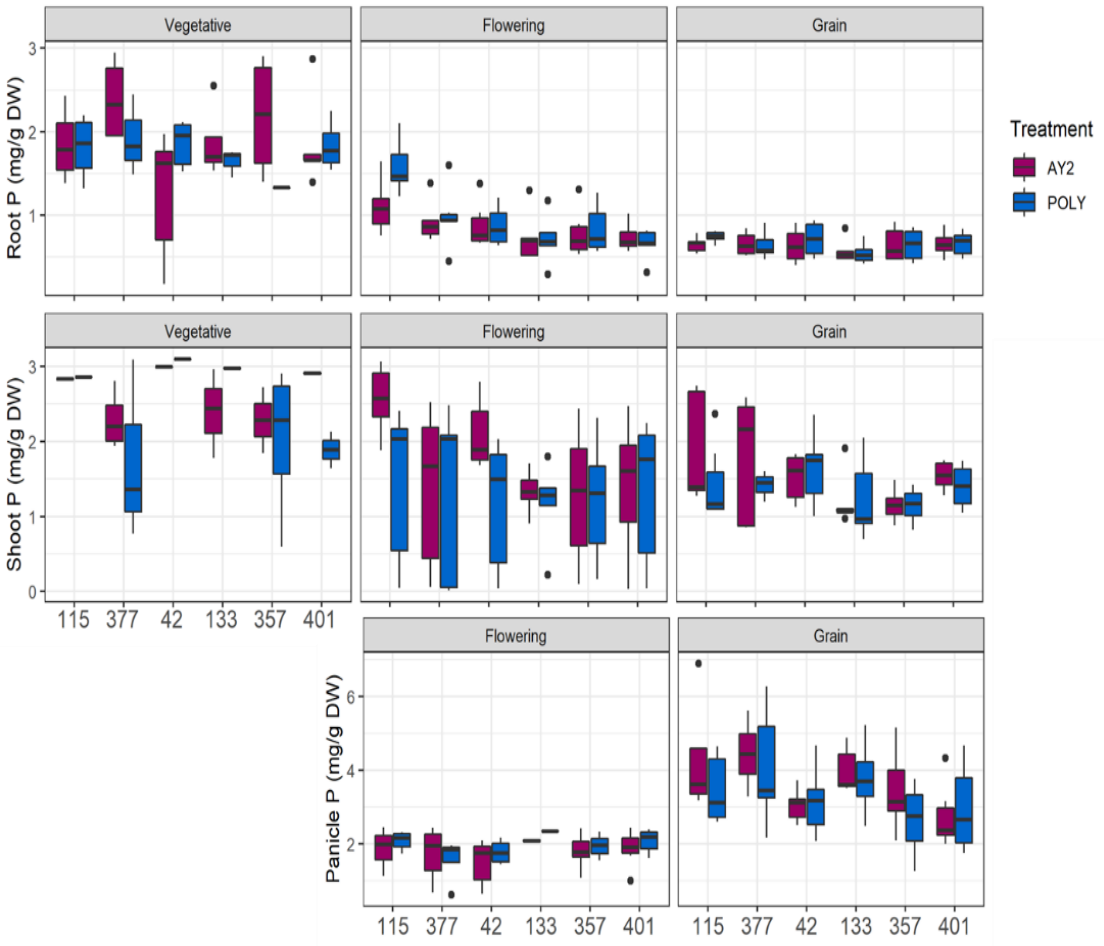

Figure 6.2.5 – Root, shoot and panicle P content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences (($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

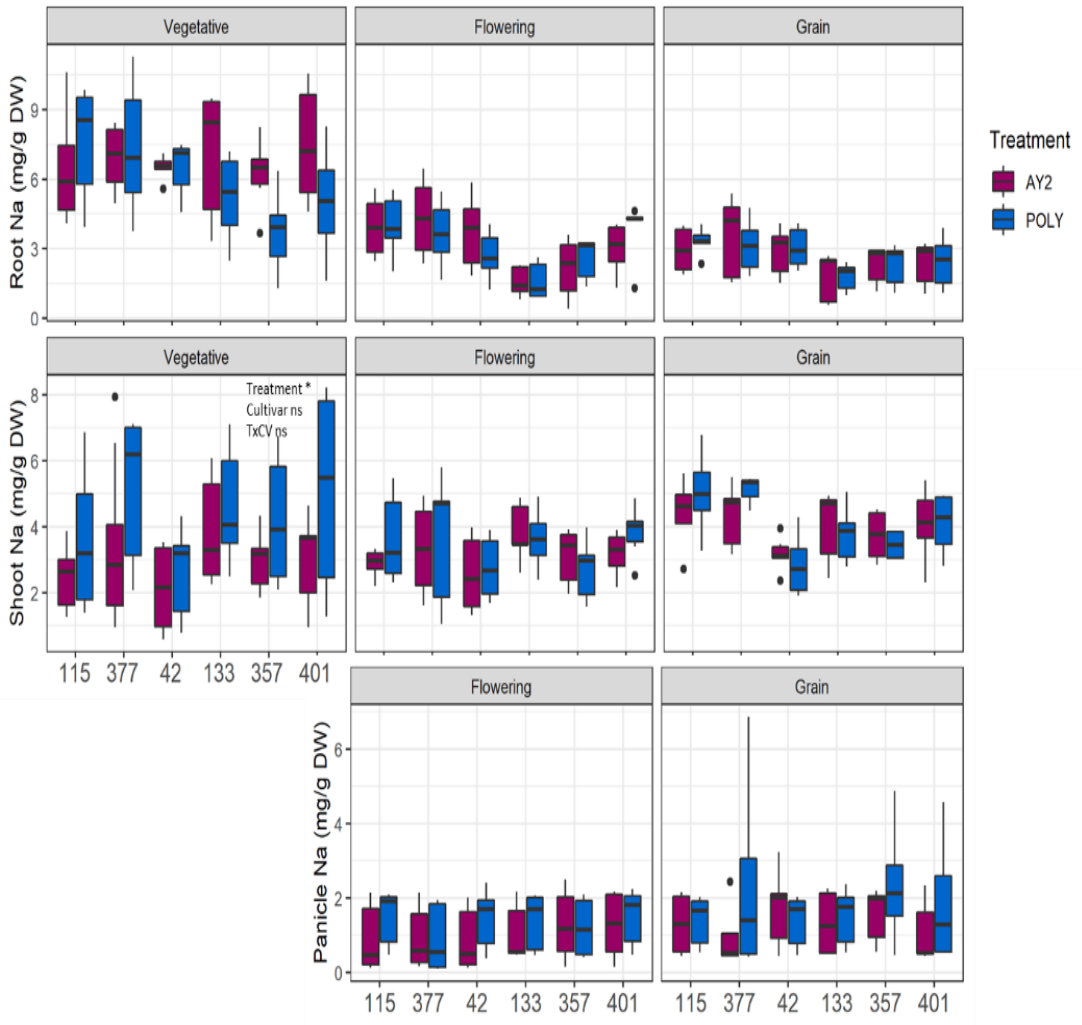

Figure 6.2.6 – Root, shoot and panicle Na content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences (($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

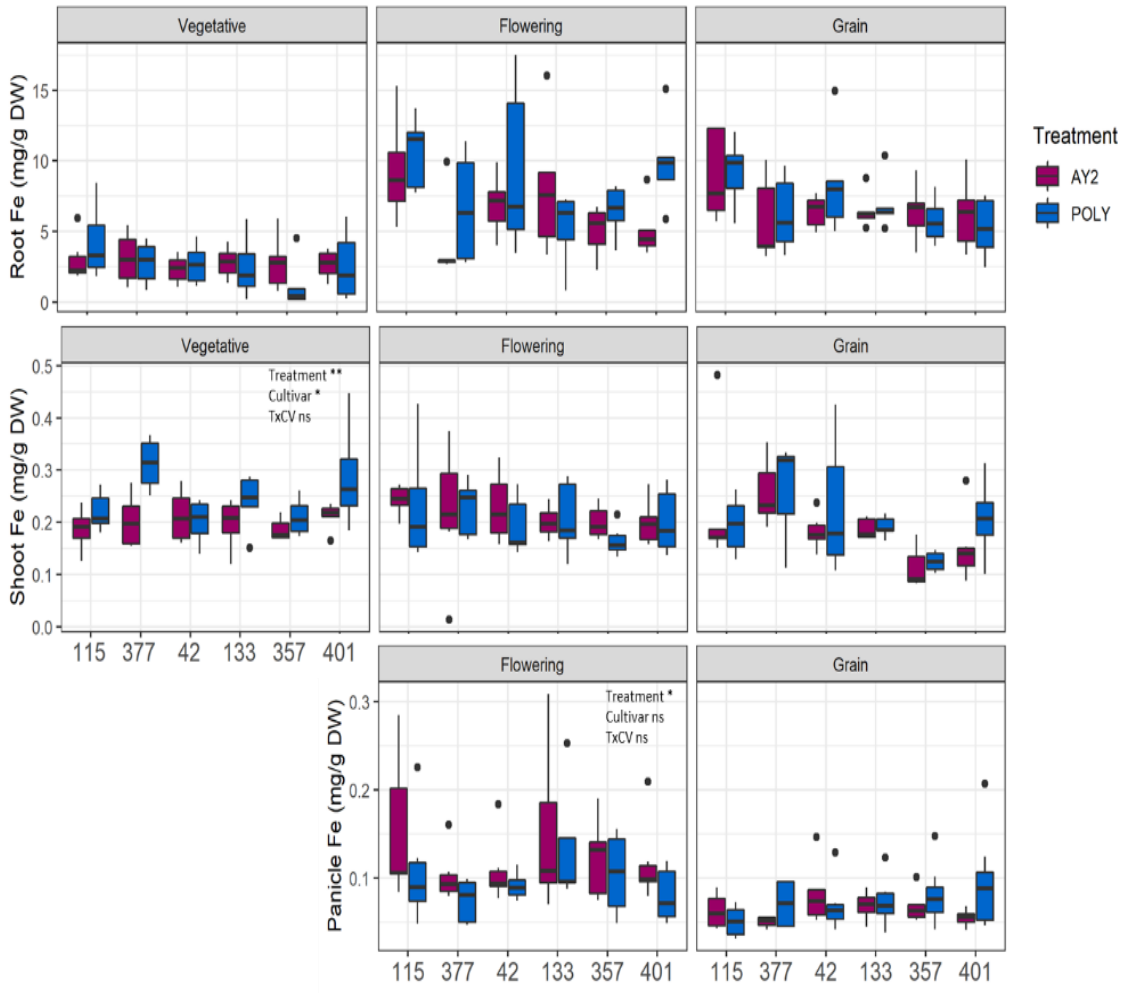

Figure 6.2.7 – Root, shoot and panicle Fe content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences ($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

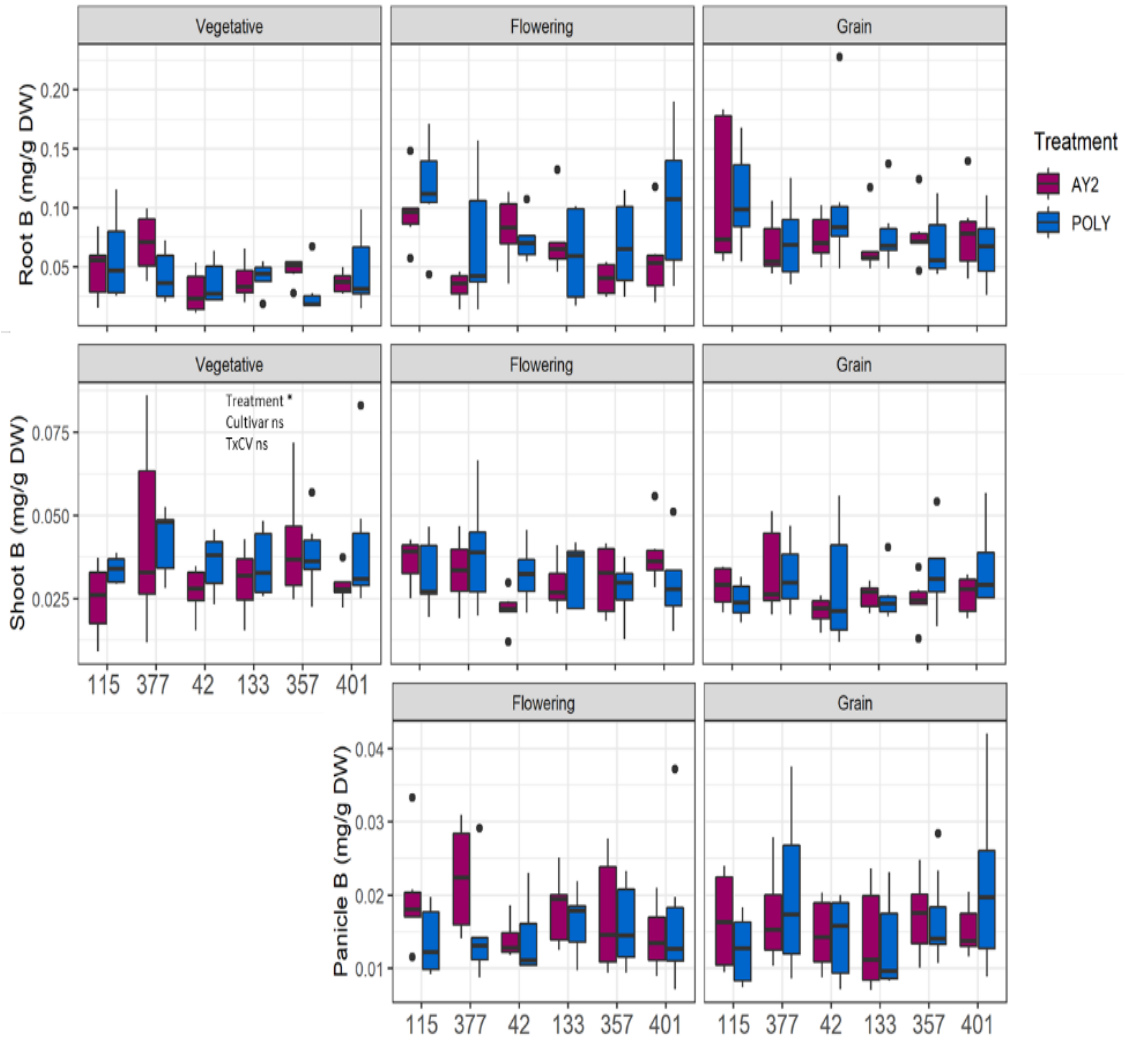

Figure 6.2.8 – Root, shoot and panicle B content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences (($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

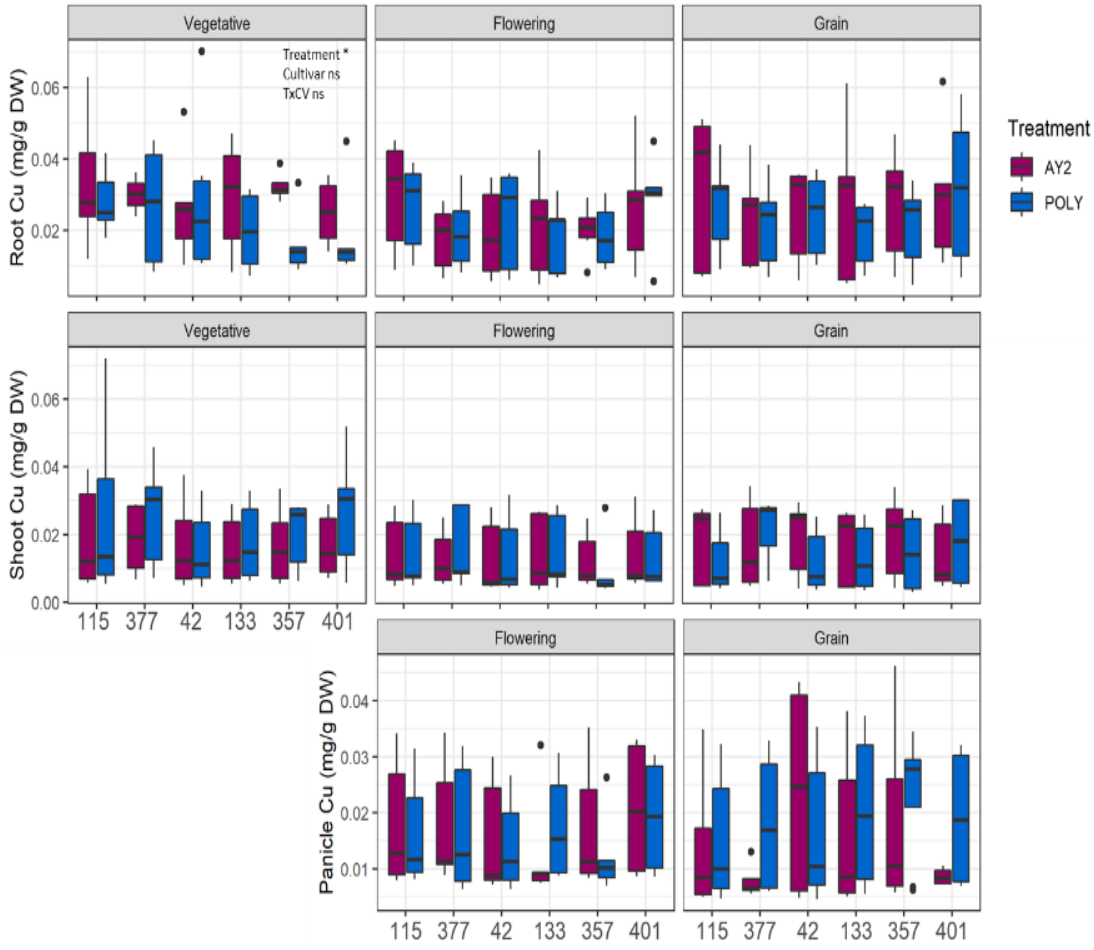

Figure 6.2.9 – Root, shoot and panicle Cu content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences (($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

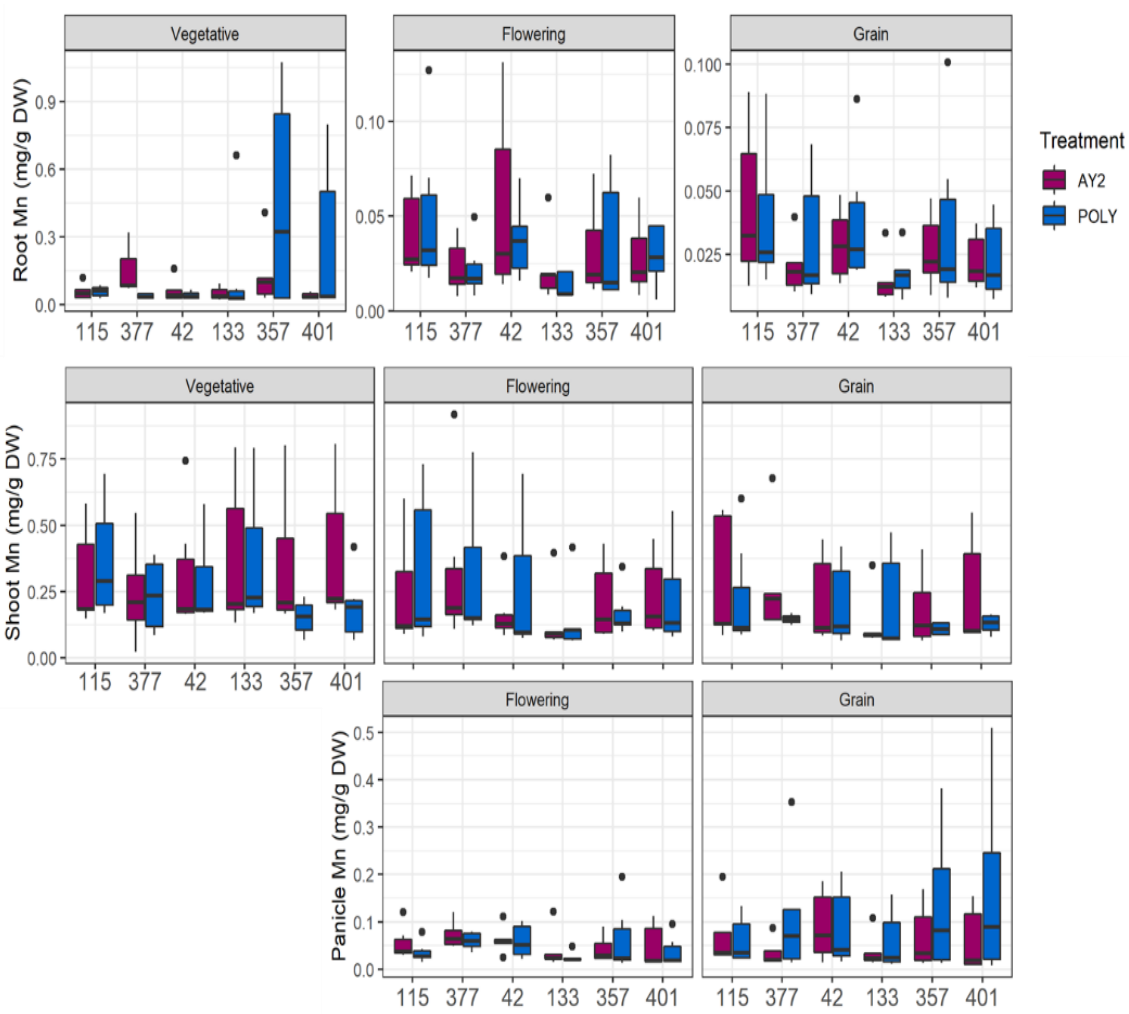

Figure 6.2.10 – Root, shoot and panicle Mn content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences ((p < 0.05) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* p < 0.05 ** p < 0.01 *** p < 0.001) in the top right corner of each plot. Each boxplot represents twelve plants.

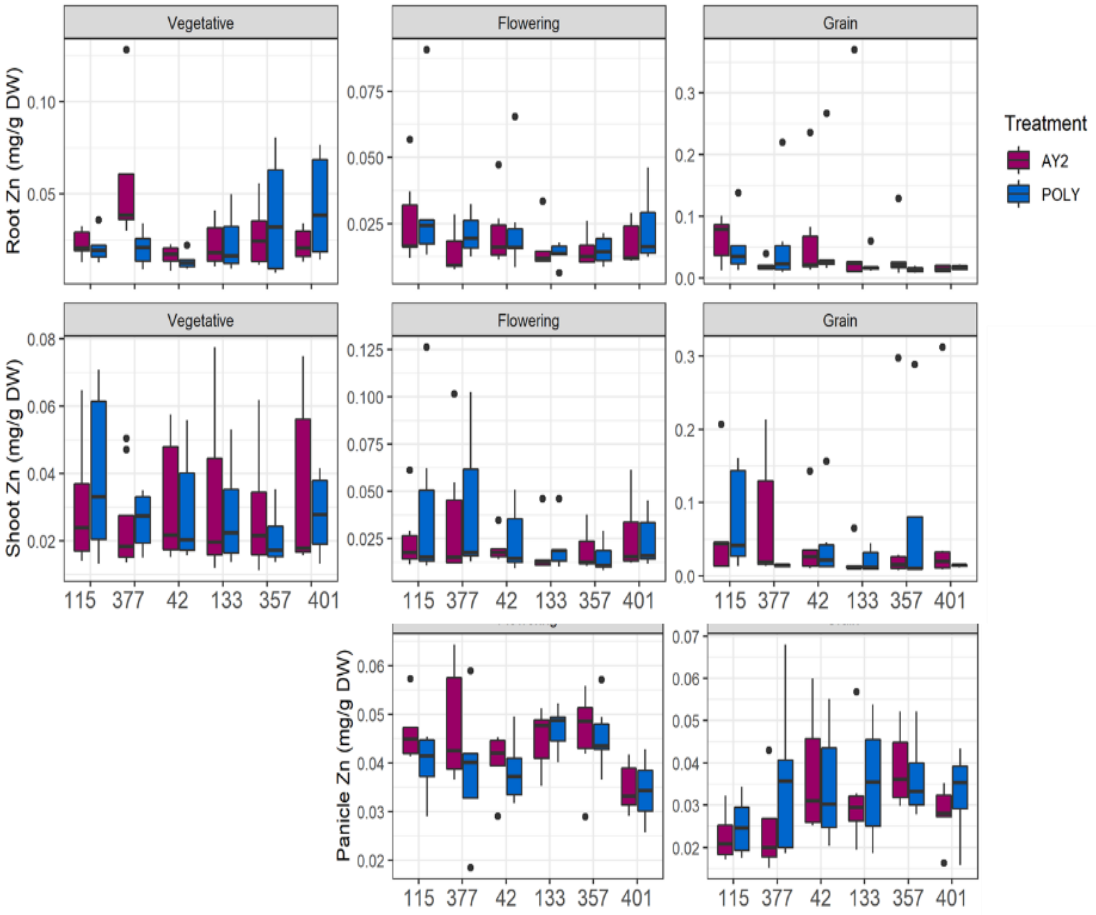

Figure 6.2.11 – Root, shoot and panicle Zn content at the vegetative, flowering and grain stages of six rice cultivars. See fig.2.1. caption for explanation of boxplot. Significant differences (($p < 0.05$) ANOVA followed by Tukey post-hoc test or Kruskal Wallis test followed by a Dunn's post-hoc test) between treatments, cultivar (CV) or interaction between treatment and CV (T x CV) are indicated with treatments are indicated with asterisks (* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$) in the top right corner of each plot. Each boxplot represents twelve plants.

6.3 Supplementary figures for Chapter 4

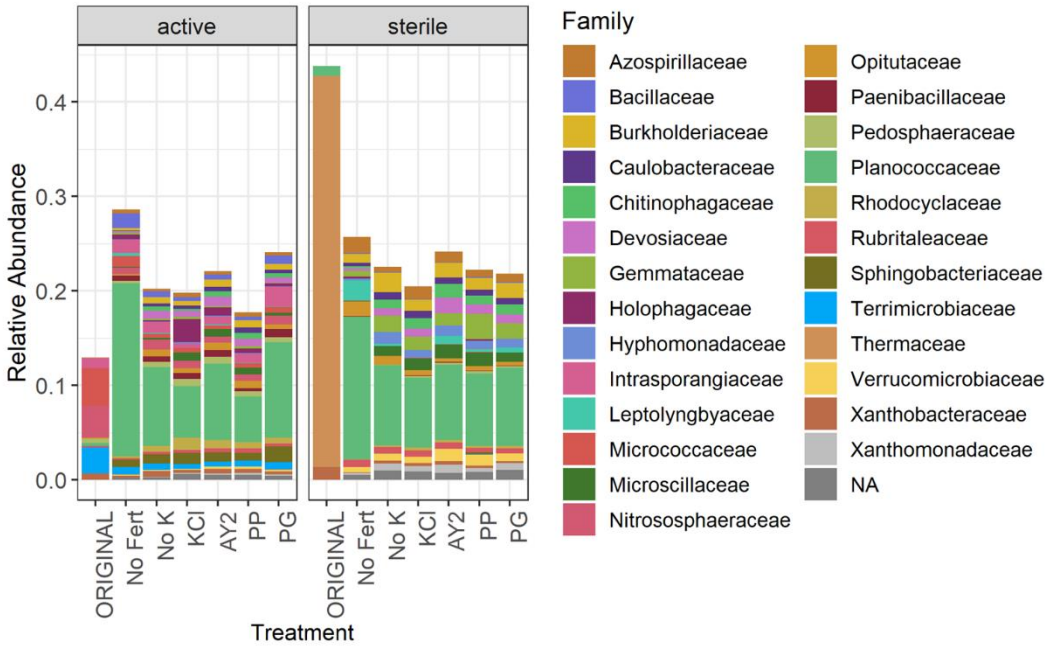

Figure 6.3.1 - Relative abundance of the families from the top 50 taxa of soil bacterial community populations from plants grown in unbalanced K fertiliser regimes. Stacked barplots of the mean relative abundance of the top 50 taxa grouped by family of the soil bacterial community populations from the original inoculum (ORIGINAL) or plants grown with the following fertiliser treatments; no fertiliser (No Fert), N, P and micronutrient fertilisers only (No K), KCL, adjusted yoshida (AY2), polyhalite powder in solution (PP) and polyhalite granules (PG)).

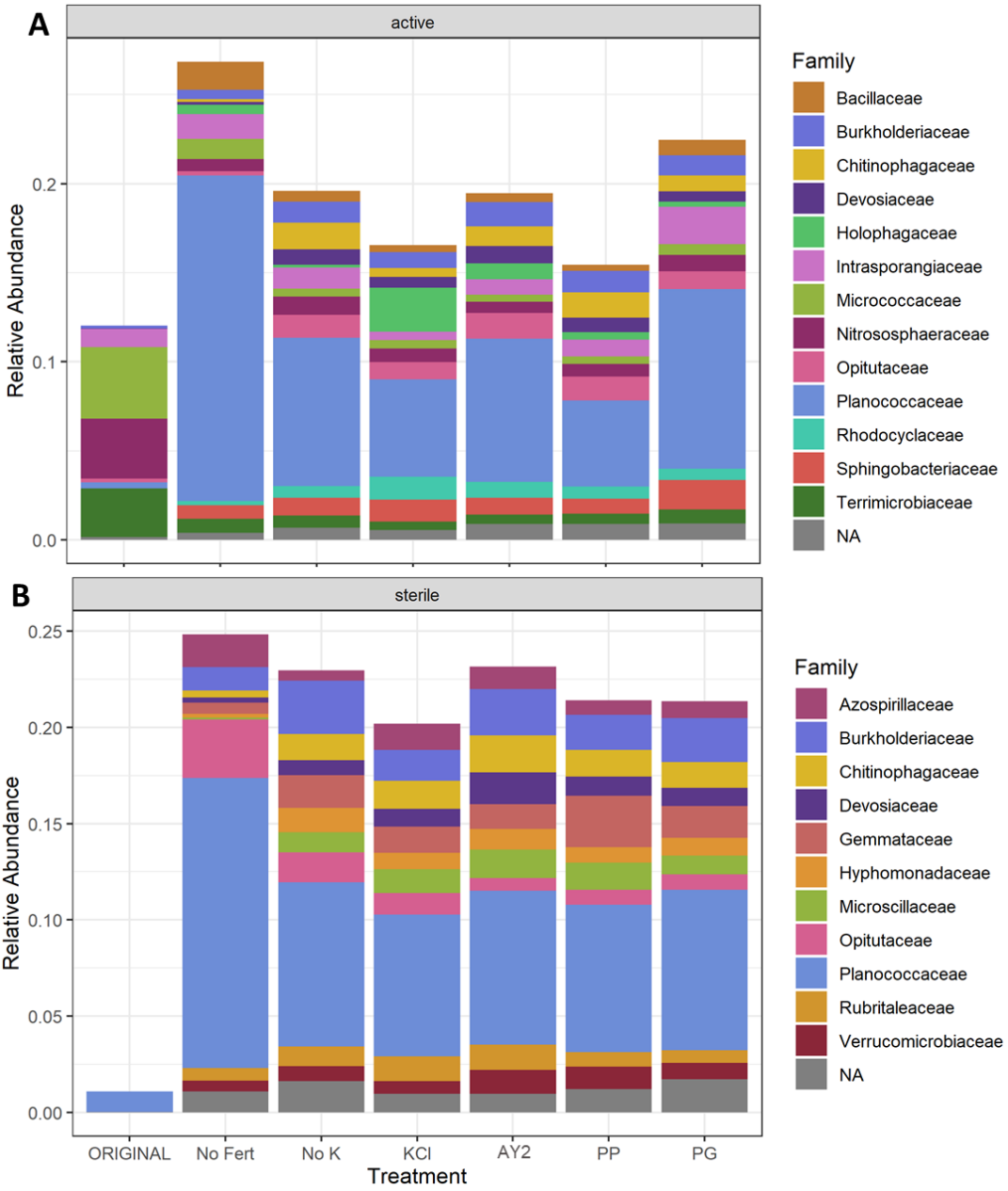

Figure 6.3.2 -Relative abundance of families of the top 20 taxa of soil bacterial community populations considering inocula separately, from plants grown in unbalanced K fertiliser regimes. Stacked barplots of the mean relative abundance of the top 20 taxa grouped by family in the active (A) and sterile (B) original (ORIGINAL) inocula of the soil bacterial community populations. Plants were grown with the following fertiliser treatments; no fertiliser (No Fert), N, P and micronutrient fertilisers only (No K), KCL, adjusted yoshida (AY2), polyhalite powder in solution (PP) and polyhalite granules (PG).

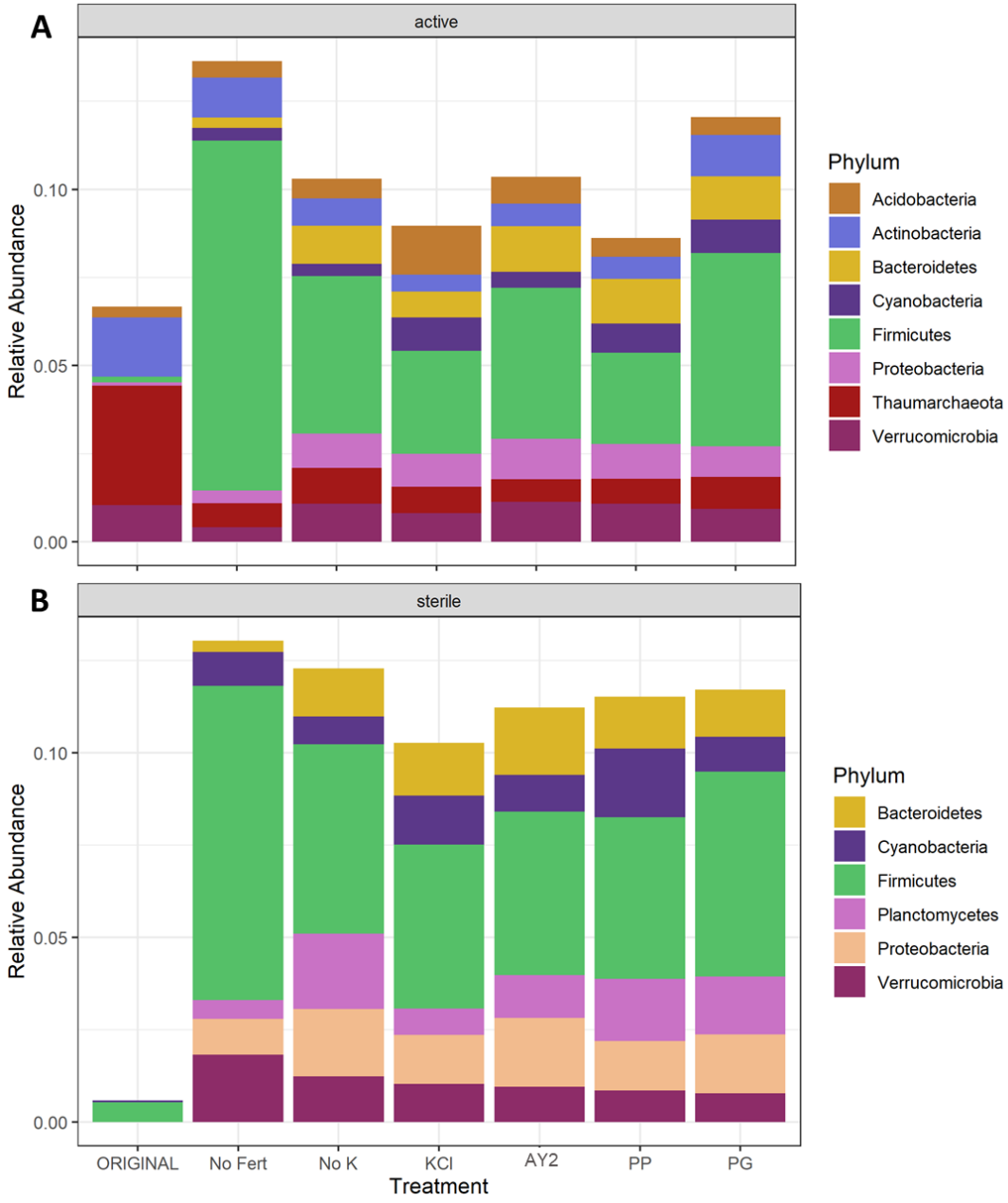

Figure 6.3.3 -Relative abundance of the Phyla of the top 20 taxa of soil bacterial community populations considering inocula separately, from plants grown in unbalanced K fertiliser regimes. Stacked barplots of the mean relative abundance of the top 20 taxa grouped by Phylum in the active (A) and sterile (B) original (ORIGINAL) inocula of the soil bacterial community populations. Plants were grown with the following fertiliser treatments; no fertiliser (No Fert), N, P and micronutrient fertilisers only (No K), KCL, adjusted yoshida (AY2), polyhalite powder in solution (PP) and polyhalite granules (PG).

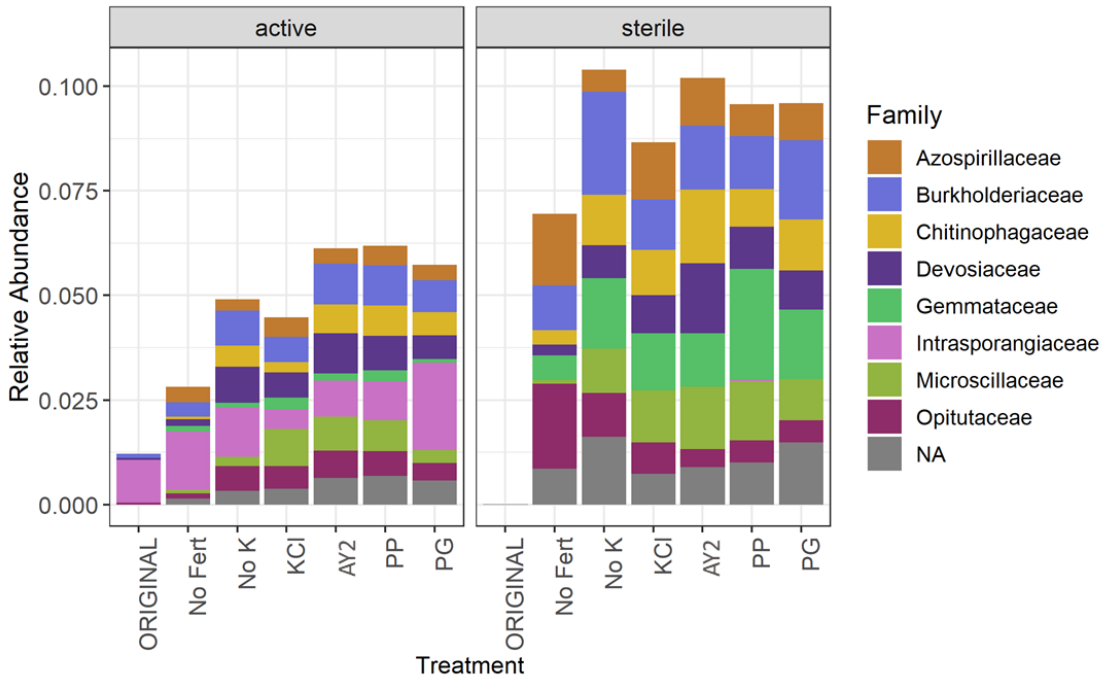

Figure 6.3.4 -Relative abundance of the families of the top 20 taxa of soil bacterial community populations considering inocula together with the *Planococcaceae* taxa removed, from plants grown in unbalanced K fertiliser regimes. Stacked barplots of the mean relative abundance of the top 20 taxa grouped by Phylum in the active (A) and sterile (B) original (ORIGINAL) inocula of the soil bacterial community populations. Plants were grown with the following fertiliser treatments; no fertiliser (No Fert), N, P and micronutrient fertilisers only (No K), KCL, adjusted yoshida (AY2), polyhalite powder in solution (PP) and polyhalite granules (PG).