PHYTOREMEDIATION OF NUTRIENT POLLUTANTS BY NATIVE PLANTS

CHEN XIANGTING, CLEO
(B.Sc. (Hons), NUS)

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Chen Xiangting, Cleo
11 March 2015

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Summary

Water security is critical in an urban city such as Singapore, and stormwater runoff can be a valuable freshwater resource. During natural precipitation, stormwater flows along impervious surfaces, washing many pollutants into nearby water bodies. Nutrient pollutants that are washed into water bodies have detrimental effects. Plants can be used to remove pollutants from water or filter media, and this is known as phytoremediation. In this study, 25 species of plants native to Singapore were studied to determine their phytoremediation potential for stormwater runoff pretreatment in bioretention systems.

The plants were monitored for 7 weeks, during which they were irrigated with a chemically spiked nutrient solution for 4 weeks after a 3 week acclimatization period. During the 7 weeks, various non-destructive analyses were conducted to determine plant health and growth patterns. These non-destructive analyses include chlorophyll fluorescence, leaf greenness, leaf length, total number of leaves, number of new leaves, and visual assessment. The soil moisture was also recorded. During the 4 weeks of nutrient solution irrigation, the effluent of each pot was collected to analyze the nitrate and phosphate concentrations, as well as the pH and conductivity. A flow rate analysis was also conducted to understand how the presence of the different plant species affected the rate of exfiltration.

After the non-destructive period, the plants were harvested and separated into different plant parts for further destructive analysis. The specific leaf area was determined, and concentrations of total soluble proteins (TSP) of the fresh leaf samples were analyzed. The dry weight of the plants

was recorded, and the root:shoot ratio was calculated. The total Kjeldahl nitrogen (TKN) and total phosphorus (TP) levels of the dried plant materials were also determined to understand the nutrient accumulation in various plant parts. After all the experimental analyses were completed, the results were analyzed to understand how the nutrient removal of different species was related to the corresponding plant traits.

Overall, there were 11 species that showed nitrate removal, 10 of which were trees species and one was a climber. *Talipariti tiliaceum* trees exhibited the highest nitrate removal (59%), followed by *Syzygium leucoxylon* (52%) trees and *Paederia foetida* (52%) climbers. Both vegetated and barren systems showed the same phosphate removal trend, approximately 100%. Phosphate removal was not significantly different from the soil for all plant species as one of the predominant phosphorus removal mechanisms was adsorption to the soil particles. Nitrogen accumulation in the aboveground organs was detected in 3 tree species, and phosphorus accumulation was detected in the aboveground organs of another 3 tree species. Two species of large shrubs or small trees showed phosphorus accumulation in the roots. The correlation analysis showed that root dry weight and total plant dry weight were strongly correlated to nitrate and phosphate removal in tree species. Root thickness in tree species showed a statistically significant relationship to nitrate and phosphate removal as well. And lastly, the total plant dry weight of non-tree species showed a significant relationship to flow rate.

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Chapter 1. Introduction

Singapore, like any other urban city, is water scarce. The need for water security in Singapore is high. With research and technology, as well as strategic urban planning, Singapore has managed to solve her water challenges through six channels — local catchment water, imported water, NEWater, desalinated water, reservoirs in the city, and the used water superhighway (PUB, 2013). With the construction of the Marina, Punggol, and Serangoon Reservoirs in 2011, Singapore has increased its catchment area from half to two-thirds the land surface area (PUB, 2013). With such a large catchment area, it is important that Singapore maintains high standards of water quality in these catchment areas and reservoirs. With such a large-scale collection of urban stormwater in the reservoirs, it is important to put in place systems that protect the water quality from the non-point source pollution risks associated with stormwater, as these water supplies will eventually be used to supply drinking water to Singapore's residents.

Important pollutants that can be found in significant amounts in stormwater runoff in Singapore are nutrients such as nitrogen (N) and phosphorus (P) (Chui, 1997; Lim, 2003; Brydon et al., 2006; Joshi and Balasubramanian, 2010). These nutrient pollutants may be introduced into the stormwater system by organic matter, fertilizers, compost, animal waste, leaky sewage infrastructure, and gaseous N (nitric and nitrous oxides) from motor vehicle exhaust (Wong et al., 2000). Singapore prides herself as a garden city and her many parks and gardens are often fertilized. These fertilizers contain high amounts of nitrate and

phosphate, essential macronutrients for plant growth and reproduction (Taiz and Zeiger, 2002). During storm events, the excess fertilizers and compost could be washed away and carried into nearby catchments by the stormwater. Singapore does not have an agriculture industry and the manicured gardens and parks could be one major source of the excess nutrient pollutants found in the stormwater. Both nitrogen and phosphorus, especially the soluble forms, are of concern due to their eutrophic effect in waterways (Taylor et al., 2005).

In most cities, urban stormwater management was traditionally based on a drainage system that focused on the rapid collection and channeling of runoff out of the city into nearby streams or rivers (Roy et al., 2008). In Singapore, the traditional form of stormwater management is concretized canals and drains. However, these existing infrastructure and stormwater management practices are aging and there is a need to switch from a traditional "drainage city" to a "waterways city". This is an improved stormwater management initiative aimed at intercepting, attenuating, and retaining stormwater flows with the end goal of improving or maintaining the water quality and flow regime of the runoff to mimic that of pre-urban development (Bratières et al., 2008; Dietz and Clausen, 2008; Emerson and Traver, 2008; Hatt et al., 2009). One such initiative in Singapore is the Active, Beautiful, and Clean Waters (ABC Waters) program started by the Public Utilities Board (PUB) in 2006. Similar programs have also been implemented, such as the water-sensitive urban design (WSUD) in Australia, low-impact development (LID) in the United States of America, and sustainable urban drainage system (SUDS) in the United Kingdom. These urban design and

planning philosophies have similar aims – to minimize the hydrological impacts of urban development on the surrounding environment (Beecham et al., 2012). This goal is usually achieved by the incorporation of both structural water treatment devices as well as non-structural initiatives. It is a multipronged approach that integrates new and green infrastructure, such as rain gardens, with the existing canals and drains, as well as community involvement, research and development, education programs, and so on. It is also important to make such programs easy to adopt and generic enough for the different stakeholders to apply and participate.

Some examples of systems that are part of the ABC Waters program are rain gardens and bioretention swales. Such systems are multifunctional, providing the needed peak flow reduction and water quality improvement. Often, such systems have carefully selected vegetation which enhances the aesthetic value of the urban area as well as increases biodiversity (Kazemi et al., 2009). Vegetation is important for such systems as they may directly or indirectly contribute to pollutant treatment efficiency (Laurenson et al., 2013). Some examples of the direct benefits attributed to the vegetation include degradation of organic pollutants, phytoremediation of macronutrients and heavy metals, and maintenance of soil hydraulic conductivity (Archer et al., 2002; Wong, 2006; Le Coustumer et al., 2007). Heavy metal pollutants are defined as metals that have a density of more than $5gcm^{-3}$. Examples of heavy metals are cadmium, zinc, and lead, and the main threat they pose is their toxic effects on human health (Järup, 2003). In contrast, nutrient pollutants are mainly nitrogen and phosphorus and

pose more of a threat to water bodies because of their eutrophic effects. Plants also contribute indirectly through their influence on the soil microbial community by their root exudates or by altering the flow rate (Read et al., 2008). Vegetation in these landscaped bioretention areas also plays a part in slowing the surface flow and filtering sediments, thereby facilitating the physical trapping and biological uptake of the nutrients (Davis et al., 2009). Such bioretention systems aid an urban city to build resilience in its catchments and water supply.

This study aimed to elucidate the nitrate and phosphate (nutrient pollutants) phytoremediation potential of 25 plant species native to Singapore. A wide variety of different native plant species were used to allow comparison between plants of different natural habitats, growth characteristics, and morphologies. The study was conducted in a soil-based filter media to allow practical future application in bioretention systems in Singapore.

Chapter 2. Literature Review

Urbanization has increased the imperviousness of surfaces and replaced natural channels with constructed pipes, drains, or canals, disrupting the natural equilibrium of natural waterways and the hydrology of a given location (Wong et al., 2000; Walsh et al., 2005). Where precipitation was once able to infiltrate into the ground and subsequently recharged the ground water, urban areas are now being developed into impervious areas such as roads, parking lots, pavements, and roofs. This disruption to the natural hydrology causes changes such as increased frequency of stormwater flow events (Chowdhury et al., 2010). Stormwater runoff is the water from precipitation that flows over the ground surface. With the increase in volume and frequency of stormwater runoff, the frequency and magnitude of flood events would also potentially increase (Dodds et al., 2003; Walsh et al., 2005). Other impacts to the flow regime in urban environments include greater peak flow (Wang et al., 2001) and larger volume of runoff (Konrad et al., 2005). As stormwater flows over the surface of developed areas, it washes different pollutants from various anthropogenic land uses into the water bodies (Goonetilleke et al., 2005). Stormwater runoff then becomes one major nonpoint source of pollution.

The pollutants that are of concern in Singapore's stormwater are nitrate and phosphate, which have numerous negative impacts. In two broad categories, nutrient pollutants such as nitrate and phosphate can affect the environment, as well as human health, negatively. Water bodies usually support a small amount of aquatic life due to the naturally low nutrient content. However, when the water

bodies are enriched with nutrients such as nitrate and phosphate, they become eutrophic, causing an overgrowth of undesirable algae and aquatic weeds that deplete oxygen supply and would decompose when they die (Khan and Mohammad, 2014). This is known as eutrophication, where excessive nutrients in water bodies result in high production of autotrophs, including algae and cyanobacteria (Khan et al., 2014). During this eutrophication process, the high productivity in eutrophic waters leads to high respiration rates, resulting in anoxia or hypoxia and the subsequent death of many aquatic organisms (Khan et al., 2014). Eutrophic water bodies then have low light penetration, support little meaningful aquatic life, and produce a foul smell because of the decay (Beeton, 2002; Khan and Ansari, 2005; Khan et al., 2014). Singapore reservoirs are not spared from the ill effects of excess nutrients and are frequently overgrown with aquatic weeds such as *Eichhornia crassipes* and *Salvinia* spp. due to upstream nutrient inputs (Tan et al., 2010). When such water bodies are associated with human activities such as cleaning or bathing, a source of food or recreation, the water quality and/or food quality will pose substantial risks on human health (Callisto et al., 2014). Drinking water that is contaminated with nitrate is especially detrimental to human infants and is a well-known risk factor for methemoglobinemia (blue baby syndrome) among infants and young children (Knobeloch et al., 2000; Sadeq et al., 2008). Nitrate in drinking water has also been shown to be a risk factor in colon cancer (Yang et al., 2007) and thyroid disorders (Gatseva and Argirova, 2008). With such detrimental impacts on the

environment and health, it is important to protect the water quality of surface water bodies as well as ground water.

One suggested solution to the negative impacts of excess nutrients on water bodies is phytoremediation (Khan and Mohammad, 2014). Phytoremediation is the use of higher plants to decontaminate soil, water, and air in a non-invasive, cost effective way (Boyajian and Carreira, 1997; Pulford and Watson, 2003; Singh et al., 2003; Adriano et al., 2004; Robinson et al., 2009). There are several processes through which phytoremediation occurs: phytofiltration or rhizofiltration, phytoextraction, phytoimmobilization or phytostabilization, phytodegradation, and phytovolatilisation (Arthur et al., 2005). Phytofiltration is the use of plants to absorb contaminants from water such as streams (Dushenkov et al., 1995). Phytoextraction is the use of plants for removing and concentrating the pollutants in the harvestable parts of the plants (Kumar et al., 1995; Pulford and Watson, 2003). Phytoimmobilization or phytostabilization uses plants to decrease the mobility and subsequent bioavailability of the contaminants by the prevention of their migration or immobilization (Vangronsveld et al., 1995; Chen et al., 2007). Plants immobilize metals in the filter media by causing changes in the rhizosphere mainly through root exudates and their effects on the physical properties of the rhizosphere itself, microbial communities and activities, root morphology, or filter media acidification, chelation and complexation, preicipitation, and redox reactions (Bolan et al., 2011). The contaminants are released when the plants decompose after uptake, and these are immobilized in either a mineral-containing mat or a

mineral-amended soil (Arthur et al., 2005). Phytodegradation is the use of plants and any of its associated microbes to breakdown or degrade an organic pollutant to its metabolites or smaller constituents (Burken and Schnoor, 1997; Pulford and Watson, 2003; Arthur et al., 2005). Lastly, phytovolatilisation is the use of plants to volatilize the pollutants into the atmosphere (Bañuelos et al., 1997; Burken and Schnoor, 1999). This wide range of phytoremediation processes can be applied in a variety of cost effective pollutant treatments. Coupled with biotechnology such as selective breeding (Kopp et al., 2001; Bert et al., 2003) and transgenic approaches to improve plant performance for metal pollutant uptake (Berken et al., 2002; Pilon-Smits and Pilon, 2002; Tong et al., 2004), phytoremediation can become more widespread beyond the range and limitations of the plant species used (Arthur et al., 2005).

As mentioned earlier, bioretention systems function to maintain the water quality and flow regime of a developed area to that of pre-urban development (Bratières et al., 2008; Dietz and Clausen, 2008; Emerson and Traver, 2008; Hatt et al., 2009). Plants in such bioretention systems and vegetated stormwater management systems are not only important for their phytoremediation qualities, but also because of the microbial communities that are associated with them. Plant root exudates, as mentioned previously, can influence the microbial communities, activities, and structures, which may in turn mediate various biochemical transformations in the rhizosphere, including redox reactions and chemical speciation (Park et al., 2011). Both biotic and abiotic redox reactions play a part in controlling the oxidation state, mobility, and toxicity of various

metals such as arsenic, copper, chromium, lead, nickel, and selenium (Violante et al., 2010). The microbes associated with the roots of the plants may reduce certain metals to a lower redox state which is less mobile and less toxic (Laurenson et al., 2013). In addition to the root exudates, the microbes themselves are also capable of producing a number of extracellular metabolites that play a role in complexing the metals in the soil solution, including polysaccharides, pigments, siderophores and organic acids (Violante et al., 2010). The microbial cell wall can also adsorb or reduce metals via a variety of functional groups such as phosphate, carboxyl, amine, as well as phosphodiester groups (Park et al., 2011).

Plants can also influence the migration of subsurface water as transpiration results in the rapid uptake of large volumes of water from the soil (Bolan et al., 2011). This is known as hydraulic control where the plants act as natural hydraulic pumps once the plants have established a dense root network (Bolan et al., 2011). When the vegetation has a dense root system near the water table in the soil, the plants can transpire a large volume of water a day, up to 6 L of water plant^{-1} m^{-2} d^{-1} or 2190mm per year (Ashwath and Venkatraman, 2010). The highest rate of transpiration occurs in warm, uniform, tropical forest areas with 32 $\times 10^{15}$ kg y^{-1} of water vapour passing through the stomata, double the water vapour content of the atmosphere (Hetherington and Woodward, 2003). This remarkable capacity of plants have been employed to protect ground water and drinking water supplies by decreasing the migration of contaminants from the surface water downwards (Bolan et al., 2011). The hydraulic control of plants are the driving force for phytostabilization or phytocapping where a dense layer of

vegetation is grown on a layer of soil material placed on top of the contaminated site (Chen et al., 2007; Venkatraman and Ashwath, 2007). When water is trapped at the root zone and the plants take up the water, it lowers the volume of water which acts as a vehicle that carries the contaminants beyond the reach of the roots, preventing the leaching of contaminants into the groundwater (Clothier and Green, 1997).

In addition to the hydraulic control of the plants, plant growth and senescence also plays an important role in maintaining filter media structure and hydraulic conductivity (Laurenson et al., 2013). Plant roots can prevent clogging of the filter media when the roots display active root growth as this results in both macro-pore formation and maintenance (Wong, 2006).

Soil pH can also be affected by plants as fluxes in hydroxide (OH^-)/H^+ ion activity are influenced by the differential uptake of ions by the roots (Tang and Rengel, 2003). For example, during NH_4^+ uptake, H^+ ions are released as they are of equivalent net charge, thereby decreasing the rhizosphere pH (Bolan et al., 2011). Other proposed sources of soil acidification are N transformation and nitrate leaching (Bolan and Hedley, 2003). Soil acidification is important for metal ion solubility and speciation because it influences redox reaction of the metals as well as modifies the surface charge in viable charge soils (Adriano, 2001). Decreased metal adsorption results from increased soil acidity or decreased pH (Tiller, 1989). This can be attributed to the following three reasons. Firstly, soils with variable charge will have a decrease in surface negative charge as a result of a decrease in pH, reducing cation adsorption. Secondly, an increase in

acidity is likely to decrease the hydroxy species of metal cations that are adsorbed preferentially over the non-hydroxy metal cations. Lastly, a decrease in pH increases the dissolution of metal compounds, thereby increasing their concentration in the soil solution (Naidu et al., 1994).

Metals are important target pollutants which plants can phytoremediate from the environment. However, as mentioned previously, nutrient pollutants are also important target contaminants that are of concerned due to their eutrophic effect in waterways. Numerous studies have shown that vegetated bioretention systems are more effective in removing nutrient pollutants than non-vegetated ones (Hatt et al., 2007a; Henderson et al., 2007; Lucas and Greenway, 2008). Plants are important in bioretention systems to enhance nutrient removal. For nitrogen removal, the vegetation type has been shown to have a critical influence (Bratières et al., 2008) due to the root architecture and physiology that affects the different associated microbial communities and soil physiochemistry (Read et al., 2008). Higher microbial activity and larger microbial populations in the root zone contribute to enhanced nitrogen uptake and assimilation of nutrients by the plants (Henderson et al., 2007; Read et al., 2008). Root architecture and growth may also be important for creating small anaerobic pockets in the soil which support denitrification and further nitrogen removal (Laurenson et al., 2013).

Although the plant demand for phosphorus is generally lower compared to that of the requirement for nitrogen, the presence of vegetation in bioretention systems also showed improved phosphorus retention (Lucas and Greenway, 2008; Read et al., 2008). The uptake of phosphorus by plants and microbes is greater

than the portion taken up by the soil through sorption as the plants and microbes actively and rapidly absorb phosphorus, especially in low-sorbing bioretention filter media and when accompanied by mycorrhizal fungi (Bolan, 1991; Richardson et al., 2005). Although it has been shown that microbes may outcompete plants for nutrients in the rhizosphere, plant roots have greater success in removing nutrients as they have a longer lifespan and greater ability to store and translocate the absorbed nutrients (Kaye and Hart, 1997). The long lifespan of plants in comparison to microbes gives the vegetation an important role as a nutrient and heavy metal sink over time, and harvesting the vegetation from bioretention systems have been suggested as a permanent phosphorus and heavy metal removal solution (Davis et al., 2006; Hsieh et al., 2007; Muthanna et al., 2007).

Plants are important in bioretention systems for the wide variety of benefits they bring. However, plant species differ in their ability for pollutant removal (Bratières et al., 2008), and this could be attributed to how they differ physiologically, chemically, and morphologically (Read et al., 2008). Different species of plants have different root architecture, biomass, transpiration rate, and growth rate, in turn affecting the biochemistry of the soil medium and microbial community (Read et al., 2008). In Australia, *Carex appressa* was shown to be the most effective plant in removing nitrogen due to its dense root architecture (Bratières et al., 2008). This was attributed to the high surface area per volume due to the dense and fine root hairs, increasing the region of soil where the plants could absorb the nutrients (Bratières et al., 2008). Plant species that have

mycorrhizal associations coupled to the extensive root systems could increase the potential for contaminant removal for both nutrients and metals alike (Laurenson et al., 2013). In some species, arbuscular mycorrhizal fungi increase the surface for absorption in the root system of the plants they have symbiotic relationships with, giving the plants access to heavy metals and soil-derived nutrients (Bratières et al., 2008; Meier et al., 2012). In another study, it was shown that plants with fine root systems were not as favourable for maintaining filter media permeability as compared to species with thick roots such as the *Melaleuca ericifolia* (Le Coustumer et al., 2012). And yet another study showed that efficient nitrogen removal was correlated to species with long, deep roots, high root biomass, and a fast growth rate (Read et al., 2010). The inconsistency of results and differences as to which plant traits are best for phytoremediation are testament that species differ greatly and there is yet to be a single most telltale trait that would associate a plant with effective pollutant removal.

Shallow rooted plants may be less effective in nutrient pollutant removal compared to species which have deep roots that penetrate the entire filter media (Laurenson et al., 2013). When the roots are able to grow throughout the filter media, the amount of contaminants which leach out could be lower as the entire filter bed would be supported by biological activity within the root zone and by improved aeration rates (Hatt et al., 2007a). Plant size has also been suggested as a contributing factor towards improved pollutant removal, although it was only shown to account for 20% – 37% of the variation (Read et al., 2008). Accumulation of metals in plants differ possibly due to different tissue

concentrations (Muthanna et al., 2007; Sun and Davis, 2007). It has also been shown that accumulation of cadmium, copper, lead, and zinc in the above- and below-ground biomass directly paralleled loading rates, where plants that were exposed to higher loads of metals had greater accumulation compared to those that were exposed to lower loads (Sun and Davis, 2007). In the same study, Sun and Davis (2007) showed that the yield of biomass was not reduced when the metal concentration increased in the plant tissue. These studies were conducted in Maryland, the United States of America, on *Panicum virgatum*, *Kentucky-31*, and *Bromus ciliatus* and tested their phytoremediation potential on metals, but metals are different from nutrient pollutants. When a species can phytoremediate metals, the same may not be true for nutrient pollutant, and a study that was conducted in USA may not apply to Singapore where nutrient pollutants are of concern. It has been suggested that when vegetation density is increased, the higher biomass of species in an area may promote greater pollutant removal (Sun and Davis, 2007; Read et al., 2008). Species such as *Carex appressa* and *Juncus amabilis* showed high removal efficiency for N and P in Australia (Read et al., 2008; Read et al., 2010). However, the plant species used in the above mentioned experiments were all species of temperate origin, giving rise to the question of whether the results can be applied to plants of tropical origin, such as the plants native to Singapore.

When designing a stormwater management system such as the ABC Waters, WSUD, LID, or SUDS, having a mixture of species has been shown to enhance the overall contaminant removal performance because different plant species differ in their ability to remove nutrient and metal pollutants (Sun and

Davis, 2007; Bratières et al., 2008; Read et al., 2008). As improvements in design of biofilters have started to reach a plateau, species selection has been suggested as the best way to maximize pollutant removal in bioretention systems (Brisson and Chazarenc, 2009). For example, *Juncus amabilis* and *Juncus flavidus* in Australia was effective at retaining nitrogen and phosphorus, but not lead (Read et al., 2008). Having a diversity of plant species may also enhance the maintenance of the hydraulic conductivity and structure of the filter media as the different root physiologies of different plant species affect macropore formation differently (Laurenson et al., 2013). A diversity of plants in a bioretention system will also improve aesthetics and support local biodiversity in an urban area. For example, biodiversity in urban Australia was enhanced as mid-stratum vegetation layers in the bioretention swales were shown to be a favourable habitat factor for invertebrates which were active aboveground (Kazemi et al., 2011). The same study by Kazemi et al. (2011) also provided evidence that flowering species were an important feature that influenced the composition of invertebrates active aboveground. Increasing the number of flowering species also increased the abundance of flower visitor species, such as pollinators (Vergara and Badano, 2009), nectarivorous invertebrates (Dover and Sparks, 2000), and florivores (Frame, 2003; Laurenson et al., 2013).

Many of the above stated studies have provided compelling evidence that vegetation selection is of utmost importance for such bioretention systems. However, the species in the studies mentioned above are all not native to Singapore. Plant-soil interactions have been known to influence soil structure,

hydrologic processes, and nutrient cycling (Ehrenfeld, 2001). However, these interactions are not well understood in the bioretention cell environment. Furthermore, even less is known about the plant-soil interactions in tropical plants in urban areas. Vegetation, soil, and climate are all interrelated (Ugolini and Spaltenstein, 1992; Ehrenfeld, 2001). Under different climatic conditions, different types of plant species can be sustained. These different plant species have different characteristics and patterns of root production that influence the production of organic acids, distribution of soil matter, and so on, which in turn shape the properties of the soil. For example, a temperate forest would differ from a tropical rainforest in its temperature, moisture availability, vegetation, and soil profile. These studies all point to the need to study plant and soil interactions in bioretention systems in the context of Singapore.

Tree species have extensive root systems as well as large biomass. A tree's large biomass, above and below ground, makes it a strategic method for the phytoremediation of metal-polluted soil (Kumar et al., 2014). In addition, trees are generally long-lived and have a long growing period. Tree species may be preferred for phytoremediation compared to annual crop because of their large biomass, root system, and long growing season (Dhillon et al., 2008). These characteristics of trees may contribute to the phytoimmobilization of large quantities of metals within the plant tissues, reducing the negative impacts of the metal pollutants on the environment (Domínguez et al., 2009; Jensen et al., 2009). For example, an age-dependent study on *Chengiopanax sciadophylloides* showed that older trees had higher accumulation of manganese compared to young trees

(Mizuno et al., 2008), showing that planting a tree species when it is young and giving it time to mature in the contaminated site will allow for long-term phytoremediation that will improve over time. Some tree species such as the eucalyptus and poplar species also have characteristics such fast growth rate which is deemed suitable for phytoremediation (Dhillon et al., 2008). In addition to the advantages of biomass and growth, it has also been shown through various studies that trees have a higher tolerance for metals compared to shrubs and herbs, showing less toxic effects at higher concentrations in comparison (Barbosa et al., 2007; Yu et al., 2008; Rocha et al., 2009; Buendia-Gonzalez et al., 2010; Shukla et al., 2011; Tripathi et al., 2012). Trees have a vast untapped potential for phytoremediation for nutrient pollutants as many of the studies conducted so far have been on metal phytoremediation. The effectiveness of using tree species to remove nutrient pollutants is still undocumented, and it is important to study the potential for nitrogen and phosphorus removal. As mentioned earlier, nutrient pollutants are of concern for their eutrophic tendencies in water bodies. Many studies have also been conducted using hydroponics and it is imperative that the studies on trees also be conducted in filter media, the media in which the trees would be grown in bioretention systems.

Chapter 3. Materials and Methods

3.1 Experimental set up

This study was conducted in the Native Plant Nursery in the Kent Ridge Campus of National University of Singapore. Plant samples were also collected for experimental investigations in the laboratory.

3.1.1 Plant materials

A total of 25 different plant species, native to Singapore, were used in the experiments. Nineteen species of the plants studied were seedlings of tree species. Although the age of the purchased tree seedlings was not known, the different species of tree seedlings were all in the range of 0.5 – 2.0m height. The seedlings of each tree species studied were of similar size. The plant species and their respective growth forms, vascular traits, and natural habitats are listed in Table 1. The plant species chosen for this study included a wide variety of angiosperms, including monocots, eudicots, herbaceous, and woody plants. These species were chosen as they represented a diverse variety of native angiosperms which were easily available from local nurseries as well as Malaysian nurseries. The plants were purchased from two nurseries. Hua Hng Trading Company Private Limited was the local nursery, and Perniagaan Tunas Harapan was the Malaysia nursery, from which some tree seedlings were purchased from.

3.1.2 Growth conditions and establishment

A few days after the plants were delivered, they were transferred from the original bags of planting media into pots filled with a sandy loam mixture. The filter medium used for planting was a sandy loam mixture of top soil, compost, and sand in the ratio of 3:2:7. Ten individuals of each species were purchased for the experiments. The pots used for planting were 200mm in height and 280mm in diameter. Some of the tree species purchased had a larger root ball; thus these species were planted in larger pots with dimensions of 600mm height and 430mm diameter. The plants were grown under natural conditions with a transparent tentage to exclude rain but allow natural sunlight to enter. The air temperature and humidity under the transparent tentage where the plants were grown were recorded weekly using the Digital Hygro-Thermometer J411-TH (Swastik Scientific Company, India). The instrument was placed on a bench at the same level at which the plants were grown. The air temperature and air humidity remained constant with small standard errors (Figure 1). Light intensity was in the range of 12 – 928 $\mu mol\ m^{-1}s^{-1}$ and varied greatly throughout a day with cloud cover.

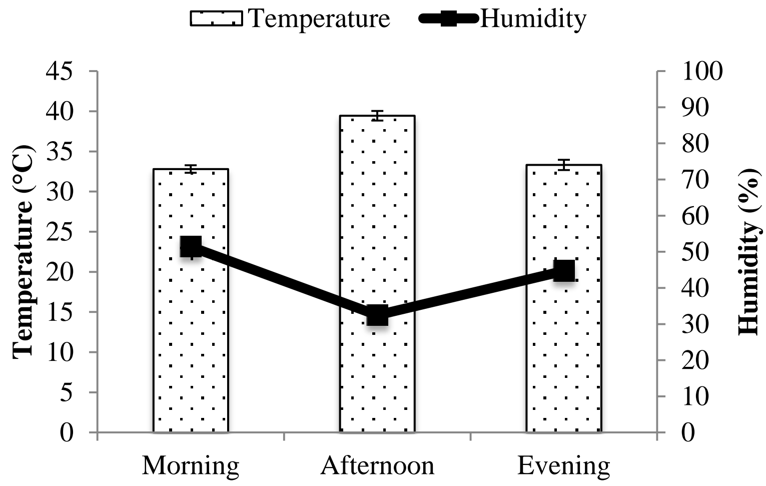

Figure 1. The mean air temperature and air humidity recorded weekly during the experimental period. The error bars represent the standard errors.

Table 1. Species used in the experiments and their growth form, vascular trait, and natural habitat. "*" refers to species planted in larger pots.

Scientific name	**Growth form**	**Vascular trait**	**Natural habitat**
Baccaurea minor Hook. f.	Tree	Eudicot	Primary forest
Barringtonia asiatica (L.) Kurz*	Tree	Eudicot	Coastal
Bhesa paniculata Arn.	Tree	Eudicot	Secondary forest, swamp forest
Bhesa robusta (Roxb.) Ding Hou	Tree	Eudicot	Lowland forest
Callicarpa longifolia Lam.	Shrub / Tree	Eudicot	Secondary forest
Cleistanthus sumatranus (Miq.) Müll.Arg.	Tree	Eudicot	Primary forest, secondary forest
Cheilocostus speciosus (J. König) C. Specht	Shrub	Monocot	Forest edge
Crinum asiaticum L.	Bulb	Monocot	Coastal
Dipterocarpus kerrii King	Tree	Eudicot	Lowland forest
Elateriospermum tapos Blume	Tree	Eudicot	Primary forest, secondary forest, forest edge
Gardenia tubifera Wall. ex Roxb.*	Tree	Eudicot	Secondary forest, swamp forest
Hopea ferrea Laness.	Tree	Eudicot	Limestone
Lithocarpus sundaicus (Blume) Rehder*	Tree	Eudicot	Primary forest, submontane forest
Paederia foetida L.	Vine	Eudicot	Primary forest, secondary forest, forest edge
Piper sarmentosum Roxb.	Herb	Eudicot	Primary forest, secondary forest
Planchonella obovata (R. Br.) Pierre	Tree	Eudicot	Coastal, limestone
Premna serratifolia L.	Shrub / Tree	Eudicot	Secondary forest, montane forest
Schefflera elliptica (Blume) Harms	Shrub	Eudicot	Primary forest, secondary forest
Sterculia macrophylla Vent.	Tree	Eudicot	Swamp forest
Syzygium leucoxylon Korth.	Tree	Eudicot	Coastal
Syzygium myrtifolium Walp.	Tree	Eudicot	Coastal
Talipariti tiliaceum (L.) Fryxell*	Tree	Eudicot	Coastal
Tarenna odorata (Roxb.) B. L. Rob.	Shrub / Tree	Eudicot	Lowland forest, swamp forest
Tristaniopsis whiteana (Griff.) Peter G. Wilson & J. T. Waterh.	Tree	Eudicot	Lowland forest
Tristellateia australasiae A. Rich.	Woody liana	Eudicot	Coastal

3.1.3 Experimental timeline and watering regime

During the non-destructive phase of the experiments, the plants were divided into batches of 6 species per batch for investigation, due to space constrains. After transplanting, all plants were grown for 3 weeks to acclimatize and establish in the new growth conditions in the Native Plant Nursery. During this period, all plants were irrigated every 3 – 4 days with 1.5L of tap water per small pot and 3L of tap water per large pot to ensure the plants were healthy before the start of the experiments. After the initial 3 weeks of establishment, the plants were randomly split into 2 groups of 5 individuals, one group to continue irrigation with tap water (plant control), and another group to start irrigation with tap water chemically spiked with additional 10mg/L nitrate (NO_3^-) and 2mg/L phosphate (PO_4^{3-}). The nitrate and phosphate solutions were made by dissolving potassium nitrate (KNO_3) and potassium dihydrogen phosphate (KH_2PO_4) in water respectively. These concentrations were chosen as they were representative of nitrate and phosphate levels commonly found in storm water runoff in Singapore (Chui, 1997; Lim, 2003; Chua et al., 2009; Joshi and Balasubramanian, 2010). The irrigation regime for the subsequent 4 weeks of treatment period remained the same as the establishment period, with the control plants irrigated every 3 – 4 days with tap water, and the treatment plants (referred to from here on as 'N10 plants') irrigated every 3 – 4 days with the solution spiked with 10mg/L nitrate and 2mg/L phosphate (referred to from here on as 'N10 solution'). Each time the pots were irrigated, the volume of tap water or N10 solution used was1.5L per small pot and 3L per large pot. Non-vegetated pots of soil without plants of both small and large pots were also irrigated and monitored according to the

same regime to control for the effect of the soil on water quality. Pots of non-vegetated soil irrigated with tap water are referred to from here on as 'control soil' and pots of non-vegetated soil irrigated with N10 solution are referred to from here on as 'N10 soil'. When a species of plant was potted with coconut fiber in the original bag of soil, it was difficult to remove all the coconut fibers from the roots of the plants without extensive damage to the root ball, as the roots would grow into the fibers and form a dense mesh. In such situations, corresponding amounts of coconut fibers were added to the soil control to make the control accurate. Plants which were potted with coconut fiber due to the above mentioned circumstances are *Pae. foetida*, *Pre. serratifolia*, and *Tar. odorata*.

3.2 Non-destructive monitoring

During the 3 weeks of establishment, as well as the subsequent 4 weeks of experiments with the N10 solution, various non-destructive tests were conducted to monitor the health and growth rate of the plants in response to different treatments. The frequency of each test will be described in greater detail in the relevant sections.

3.2.1 Chlorophyll fluorescence

Two instruments were used to determine the chlorophyll fluorescence, employed to analyze plant stress in terms of the quantification of fluorescence re-emitted by green leaves (Björkman and Demmig, 1987; Maxwell and Johnson, 2000). The leaf selected to record the chlorophyll fluorescence was the young mature leaf of each individual plant or of the branch randomly

chosen for study for plants with many shoots. The first instrument used was the Teaching PAM-210 Chlorophyll Fluorometer (Walz, Germany), which was a portable hand-held device, used to record values of F_o, F_m, and F_v/F_m value after 30 minutes of dark adaptation. The F_v/F_m value refers to the maximum quantum yield of photosystem 2 (PSII) in the dark adapted state, and it is calculated in the following formula (Maxwell and Johnson, 2000):

$F_v/F_m = (F_m - F_o) / F_m$

where,

F_o – Minimum fluorescence

F_m – Maximum fluorescence

F_v – Variable fluorescence

This instrument was used weekly during acclimatization (3 weeks) as well as the experimental period (4 weeks) on *Bar. asiatica*, *Cal. longifolia*, *Cle. sumatranus*, *Cri. asiaticum*, *Ela. tapos*, *Gar. tubifera*, *Lit. sundaicus*, *Pae. foetida*, *Pla. obovata*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, and *Tristan. whiteana* plants to monitor plant stress.

The second instrument used to determine chlorophyll fluorescence was the Fluorescence Monitoring System 2 (FMS2) (Hanstech Instruments, United Kingdom). The following parameters could be recorded by this instrument after 30 minutes of dark adaptation to give a more detailed analysis of plant stress in the dark adapted state (Maxwell and Johnson, 2000):

F_v/F_m – Maximum quantum yield of PSII

F_v'/F_m' – Antennae efficiency of PSII

Φ_{PSII} – Quantum efficiency of PSII

qP – Photochemical quenching co-efficient

NPQ – Alternative definition of non-photochemical quenching

Because each FMS2 measurement required 30 minutes to complete, the number of species which were analyzed using this instrument were limited. The FMS2 chlorophyll fluorometer was used to monitor plant stress in *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Dip. kerrii*, *Hop. ferrea*, *Pip. sarmentosum*, *Pre. serratifolia*, *Sch. elliptica*, *Tar. odorata*, and *Tristel. australasiae*, randomly chosen species. This instrument was used to monitor chlorophyll fluorescence on the above mentioned plant species weekly during establishment as well as the treatment period, a total of 7 weeks. Chlorophyll fluorescence is a sensitive indicator of stress and healthy plants would typically have a F_v/F_m value in the range of 0.75 – 0.85 (Björkman and Demmig, 1987; Maxwell and Johnson, 2000; Gorbe and Calatayud, 2012).

3.2.2 SPAD

The greenness of the young mature leaves of each plant was determined using the SPAD-502 Plus leaf chlorophyll meter (Konica Minolta, Japan). The SPAD value recorded was an average of 5 different young mature leaves of each individual plant. This was a non-destructive measurement of leaf greenness, which correlated well to chlorophyll concentration (Section 4.11). The SPAD values were recorded weekly for all 7 weeks.

3.2.3 Leaf length

The length of the young mature leaf selected for non-destructive monitoring was measured using a ruler at the start of the establishment (week 1), the start of treatment (week 4), and the end of treatment (week 7).

3.2.4 Total number of leaves and the number of leaves on a growing branch

The total number of leaves of each plant was counted on weeks 1, 4, and 7. This was to determine if the plants were shedding leaves due to the stress of transplanting, or if the plant was growing and producing more leaves. The total number of leaves was not counted for every species. If a plant species had too many leaves per plant (>150), the total number of leaves on a selected growing branch was counted instead. This was because the total number of leaves could not be counted accurately when the total number of leaves on the plant was too numerous.

A branch with a growing apical meristem was selected and the number of leaves on that branch was counted on weeks 1, 4, and 7. This parameter was recorded for all species and used to determine how many new leaves the growing branch produced over time.

3.2.5 Visual assessment

The above-ground parts of the plants were observed weekly for all 7 weeks for signs of plant stress and the degrees of wilt, burn, and yellowing of the plants were given a score according to Table 2.

Table 2. The scoring chart of the degrees of wilt, burn, and yellowing of the plants.

Wilting

0	Plant mortality
1	Over 65% of the plant wilted
2	35-65% of the plant wilted
3	Up to 35% of the plant wilted
4	Only a few leaves wilted
5	Plant fully turgid

Burn

0	Plant mortality
1	Over 50% of the leaf area burnt
2	25-50% of the leaf area burnt
3	Up to 25% of the leaf area burnt
4	Minimal burn, seen mostly on tips or edges of leaves
5	Plant showed no burns

Yellowing

0	Plant mortality
1	Over 50% of the plant yellowed
2	25-50% of the plant yellowed
3	Up to 25% of the plant yellowed
4	Only a few leaves yellowed
5	Plant showed no yellowing

3.2.6 Soil moisture

The soil moisture of all non-vegetated pots as well as vegetated pots was determined weekly at a 5cm depth using the EC-5 Volumetric Water Content Sensor (Decagon Device, United States of America). Each measurement recorded was an average of 5 points from each pot.

3.2.7 Water quality improvement analysis

Trays were placed at the bottom of the pots of the corresponding size to collect the leachates from the pots after irrigation only during the treatment period (Plate 1). All leachates were collected from the trays within 12 – 18

hours after irrigation. This was conducted weekly. The pots and trays were placed on wooden benches or pallets to prevent any surface runoff from entering the trays and contaminating the leachates when it rained. A 10mL syringe was used to draw the leachate from the tray, and the leachate was filtered through a sterile 0.20 µm-pore sized Minisart High-Flow Syringe Filter (Satorius Biotech, USA). The filtered leachate was then collected in 15mL sterile Falcon tubes and kept at 4°C until the nitrate and phosphate concentrations were determined. Prior to irrigation, samples of the tap water as well as the prepared N10 solution were also collected in the same manner for the nitrate and phosphate concentrations to be analyzed. The nitrate and phosphate concentrations of the leachate, tap water, and N10 solution were determined by ion chromatography using the Dionex CD20 Conductivity Detector (Thermo Scientific, USA). Plant nitrate and phosphate removal were later calculated using the following equation:

Nitrate or phosphate removed by plants (mg) = A - [(PL2 - PL1) - (SL2 - SL1)]

where,

A =Amount of nitrate/phosphate in N10 solution (mg)

PL2 =Amount of nitrate/phosphate in leachate from N10 plants (mg)

PL1 = Amount of nitrate/phosphate in leachate from control plants (mg)

SL2 = Amount of nitrate/phosphate in leachate from N10 soil (mg)

SL1 = Amount of nitrate/phosphate in leachate from control soil (mg)

Plate 1. *Bar. asiatica* (A) and *Bhe. paniculata* (B) plants with the trays to collect the leachates.

3.2.8 pH and conductivity of leachate

The pH and conductivity of the leachate were also determined weekly during the treatment period using a pH Meter (Hanna Instruments, USA) and the Cond 315i Conductivity Pocket Meter (WTW, Germany) respectively.

3.2.9 Flow rate

Five out of the 10 pots of each species, as well as the non-vegetated pots, were randomly selected for flow rate analysis. Flow rate analysis was conducted at the start of treatment (week 4) and the end of treatment (week 7) to understand how the presence of the different vegetation (plant species) affected the rate of exfiltration or water flow through the pots. The pots were placed on an elevated platform with a container below to collect the exfiltrate. Tap water or N10 solution (1.5L of either) was added to each pot at time 0, and the exfiltrate was collected and volume measured using a measuring cylinder at time intervals of 1, 3, 5, 10, 15, and 30 minutes. These time intervals were selected because it was observed that exfiltration was typically completed by 30 minutes. For larger pots, 3L of either tap water or N10 solution were added to each pot according to the same time intervals. The time interval was extended until 60 minutes, as it took longer for the exfiltration to be completed in larger pots. When the exfiltration was completed, the total volume of the effluent was noted (see Figure A-1).

3.2.10 Picture of plant habit

Just before the destructive harvesting of the plants at the end of the week 7, photographs of both control and N10 plants were taken to document the plant habit. The plants were photographed against a black background.

3.3 Destructive analyses

At the end of week 7 (after the 3 weeks of establishment and the 4 weeks of treatment), the plants were harvested for destructive analyses. The plants were carefully removed from the pots and the soil washed from the roots.

3.3.1 Chlorophyll concentration in relation to SPAD

The SPAD values of leaves of varying greenness were recorded per species. Leaf discs of diameter 4mm (3 – 4 discs) were also collected from each leaf using a cork borer. The fresh weight (FW) of the leaf samples was determined using an electronic weighing balance. Next, the leaf samples were ground with 5mL 100% acetone until they were colourless. The samples were then kept in the dark for 15 minutes for the proteins to precipitate. The samples were then centrifuged at 5000rpm at 20°C for 10 minutes. The optical densities of the chlorophyll extracts were determined at 460, 645, and 663 nm using absorbance spectrophotometry. The concentrations of the photosynthetic pigments, in terms of mg chlorophylls per m^2 leaf and mg chlorophylls per g dry weight (DW) of leaf tissues were calculated using the following formulae (Marr et al., 1995):

Chl a (mg L^{-1}) = [OD_{663} × 12.7] – [OD_{645} × 2.69]

Chl b (mg L^{-1}) = [OD_{645} × 22.9] – [OD_{663} × 4.68]

Total Chl (mg L^{-1}) = [OD_{645} × 20.2] + [OD_{663} × 8.02]

Carotenoids (mg L^{-1}) = [OD_{460} × 5] – [OD_{645} × 14.87] + [OD_{663} × 2.84]

The chlorophyll concentrations were then correlated to the corresponding different SPAD values. Although this correlation had been previously shown to be non-linear for soybean (*Glycine max*) and maize (*Zea mays* L.) (Markwell et al., 1995), this relationship has yet to be studied in other species such as the ones in this study.

3.3.2 Dry weight and specific leaf area (SLA)

After the plants were harvested, they were separated into various plant parts such as the leaves, stems, roots, and reproductive organs. The plant parts were then dried at 60°C for 7 days or until constant weight. The root:shoot ratio was then calculated by adding together all the above ground biomass (leaves, stems, and reproductive organs) and divided by the below ground biomass (roots).

Five young mature leaves were also harvested from each plant and the fresh weight (FW) was determined using an electronic weighing balance. The surface area of the leaves was measured using the LI-3000C Portable Leaf Area Meter (LI-COR, USA). The leaves were then dried at 60°C for 7 days or until constant weight for the determination of dry weight (DW). The SLA of the leaves was calculated as the amount of DW in grams per cm^2. The FW:DW ratios of the 5 leaves were also recorded.

3.3.3 Total Soluble Proteins (TSP)

An extraction buffer comprising 50mM phosphate buffer (pH 7.5), 0.1mM of ethylenediaminetetraacetic acid (EDTA), 0.1% (w/v) polyvinylpyrrolidone (PVP), and 0.1% (v/v) Triton-X 100 was prepared. Fresh leaf materials (0.1g) were harvested at the end of 7 weeks from the young, mature leaf, and ground in 1 mL of extraction buffer at 4˚C. The extract was centrifuged at 5000 rpm at 4˚C for 10 min. The supernatant (60µL) was extracted and 3 ml of 20% Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, USA) were added to it and allowed to stand for 5 minutes for colour development. The absorbance at 595nm was determined by absorbance spectrophotometry. Standards were prepared using varying concentrations (0 – 2 mg mL^{-1}) of bovine serum albumin (BSA). The concentration of TSP was expressed as mg proteins per g DW.

3.3.4 Total Kjeldahl Nitrogen (TKN)

Dried plant materials (0.1 – 0.2g) were placed in 100mL digestion tubes. Roots of 0.1g were used to ensure the digest appeared clear and colourless after the completed reaction. For the other plant parts, leaves, stems, and reproductive organs, 0.2g of dried plant materials were used. Four mL of concentrated sulphuric acid H_2SO_4 (95 – 97%) were added to each sample and 1 piece of Kjeltab (1.5 g K_2SO_4 and 1.5 g Se) was added. The tubes were placed in a digestor heat block set at 350°C for 2 hours. After digestion, the samples were left to cool to room temperature (25°C). Once the samples appeared clear and colourless, the TKN concentration of each sample

was analyzed using the Kjeltec 8400 Auto Sampler System (FOSS, USA) and expressed as mg N g^{-1} DW.

3.3.5 Total Phosphorus (TP)

Dried plant materials (0.05 – 0.10g) were weighed and transferred into a 100mL digestion tube. A root sample of 0.05g was used instead of 0.1g because using greater mass typically resulted in a cloudy digest. The other plant parts such as leaves, stems, and reproductive organs required 0.1g for a clear and colourless digest. Three mL of concentrated H_2SO_4 (95 – 97%) were added to each sample and the digestion tubes were swirled carefully to ensure that all the dried plant materials were kept in the acid. Two mL of 30% hydrogen peroxide (H_2O_2) were then added and the tubes were incubated on a digestor heat block at 280˚C for 1 hour. The samples were then cooled to room temperature and 1mL of 30% H_2O_2 was added before the samples were heated again at 280˚C for 5 – 10 min until all water had evaporated. This procedure was repeated until the cooled digests appeared clear and colourless. The digests were then cooled to room temperature and diluted 100× with deionised water. The standard series was prepared using varying concentrations (0.0 – 5.0mg/L) of KH_2PO_4. One mL each of the diluted sample digests, diluted blank digests, and diluted stand series were pipette into boiling tubes. The reaction mixture (1g/L ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$, 5mM ascorbic acid $C_6H_8O_6$, 35µM potassium antimonyl tartrate $KSbC_4H_4O_7$, and 1.12% H_2SO_4) (3.8 mL) was added and the mixture was vortexed. The mixtures were allowed to stand for 1 hour for colour development, after which they were vortexed again. The absorbance was determined at 880 nm by

spectrophotometry. The phosphorus content of the dried plant materials was then expressed in mmol P kg^{-1} DW, calculated by the following formula (Schouwenburg and Walinga, 1967; Walinga, 1995):

$$0.323 \times (a - b) \times V/W$$

in which

a = Concentration of phosphorus in the diluted sample digest (mg L^{-1})

b = Concentration of phosphorus in the diluted blank digest (mg L^{-1})

V = Total volume of digest at the end of the digestion procedure (ml)

W = Weight of plant material sample (g).

3.3.6 Root characteristics

After washing the soil and other filter medium particles off the harvested plant roots, a photograph of the roots was taken to document the root characteristics.

3.4 Statistical analysis

Each datum point was presented as mean ± standard error or standard deviation. Standard error was used when there were 4 or more replicates, whereas standard deviation was used when the number of replicates was less than 4. The means were compared via the Fisher's least significant difference (LSD) test (one way ANOVA and multivariance analyses) at a 5% level of significance. Correlation analyses were conducted using simple linear regression models.

Chapter 4. Results

During the non-destructive monitoring period, one plant from each of the following groups had died after repotting: control *Bhe. paniculata*, control and N10 *Hop. ferrea*, and N10 *Syz. leucoxylon*.

4.1 Physical appearance of the plants

Before the plants were harvested at week 7, photographs were taken of the plants to document the plant habit of different species, as well as the physical appearance of plants irrigated with tap water and those irrigated with N10 solution.

Generally, the control and N10 plants did not differ much in physical appearance for *Bac. minor* (Plate 2), *Bar. asiatica* (Plate 3), *Cal. longifolia* (Plate 6), *Che. speciosus* (Plate 7), *Cle. sumatranus* (Plate 8), *Cri. asiaticum* (Plate 9), *Dip. kerrii* (Plate 10), *Ela. tapos* (Plate 11), *Gar. tubifera* (Plate 12), *Lit. sundaicus* (Plate 14), *Pae. foetida* (Plate 15), *Pip. sarmentosum* (Plate 16), *Sch. elliptica* (Plate 18), *Ste. macrophylla* (Plate 20), *Syz. myrtifolium* (Plate 22), *Tal. tiliaceum* (Plate 23), *Tristan. whiteana* (Plate 24), and *Tristel. australasiae* (Plate 26).

N10 plants of *Bhe. paniculata* (Plate 4), *Bhe. robusta* (Plate 5), *Hop. ferrea* (Plate 13), *Pre. serratifolia* (Plate 17), *Syz. leucoxylon* (Plate 21), and *Tar. odorata* (Plate 25) appeared to be taller than the control plants, and *Pla. obovata* control plants appeared to be taller than N10 plants (Plate 19).

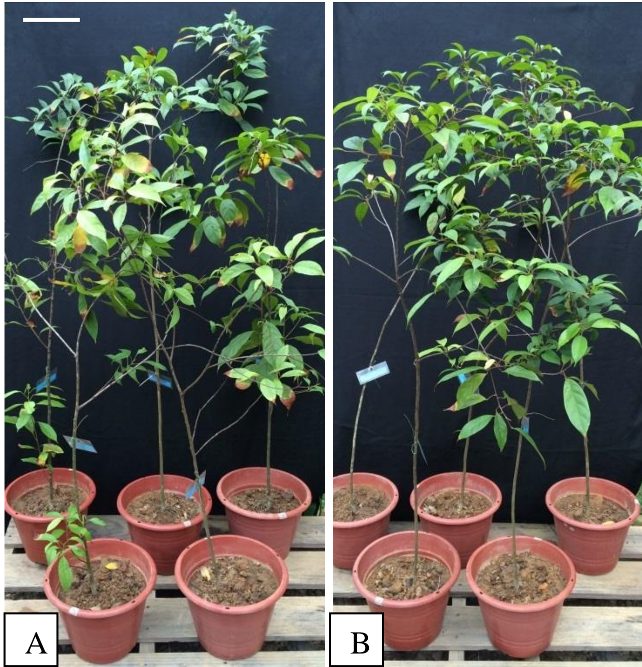

Plate 2. The 5 replicates of control *Bac. minor* plants (A) and the 5 replicates of N10 *Bac. minor* plants (B). Scale bar = 14cm.

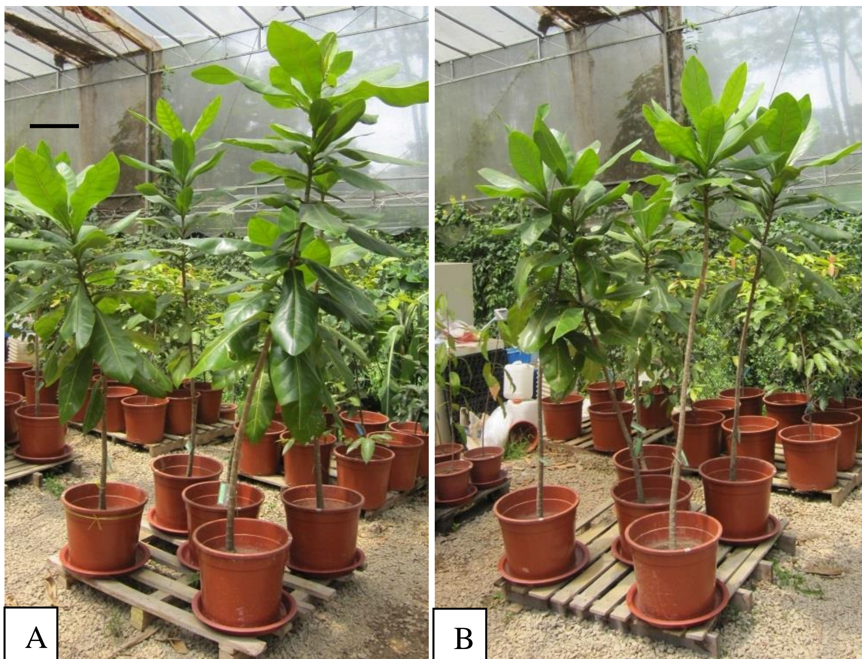

Plate 3. The 5 replicates of control *Bar. asiatica* plants (A) and the 5 replicates of N10 *Bar. asiatica* plants (B). Scale bar = 21.5cm.

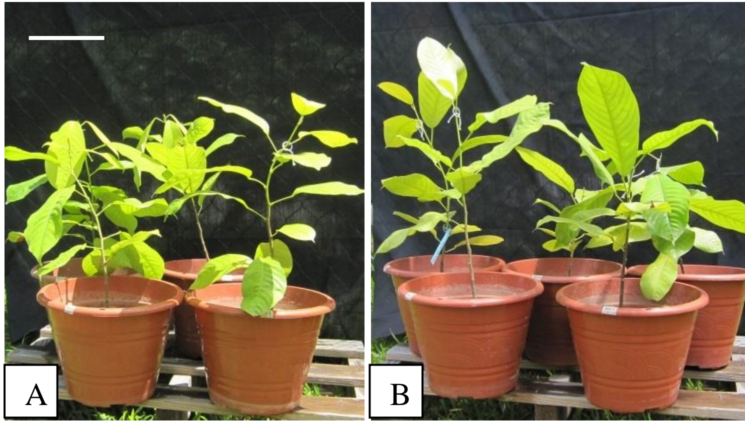

Plate 4. The 4 replicates of control *Bhe. paniculata* plants (A) and the 5 replicates of N10 *Bhe. paniculata* plants (B). Scale bar = 14cm.

Plate 5. The 5 replicates of control *Bhe. robusta* plants (A) and the 5 replicates of N10 *Bhe. robusta* plants (B). Scale bar = 14cm.

Plate 6. The 5 replicates of control *Cal. longifolia* plants (A) and the 5 replicates of N10 *Cal. longifolia* plants (B). Scale bar = 14cm.

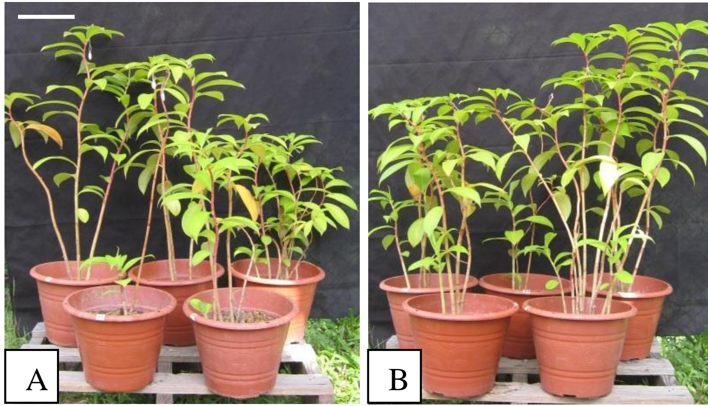

Plate 7. The 5 replicates of control *Che. speciosus* plants (A) and the 5 replicates of N10 *Che. speciosus* plants (B). Scale bar = 14cm.

Plate 8. The 5 replicates of control *Cle. sumatranus* plants (A) and the 5 replicates of N10 *Cle. sumatranus* plants (B). Scale bar = 14cm.

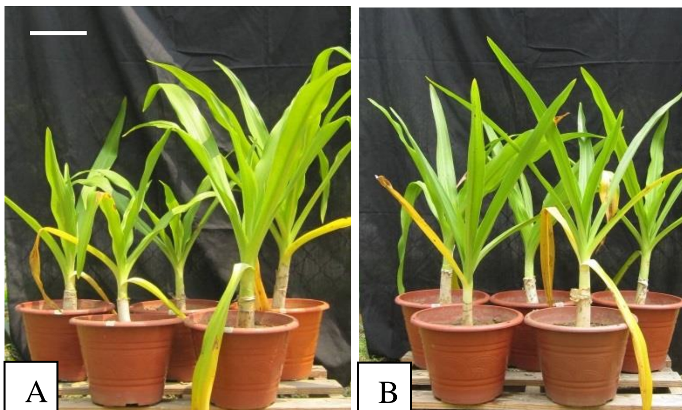

Plate 9. The 5 replicates of control *Cri. asiaticum* plants (A) and the 5 replicates of N10 *Cri. asiaticum* plants (B). Scale bar = 14cm.

Plate 10. The 5 replicates of control *Dip. kerrii* plants (A) and the 5 replicates of N10 *Dip. kerrii* plants (B). Scale bar = 14cm.

Plate 11. The 5 replicates of control *Ela. tapos* plants (A) and the 5 replicates of N10 *Ela. tapos* plants (B). Scale bar = 14cm.

Plate 12. The 5 replicates of control *Gar. tubifera* plants located in the row further from the fence and the 5 replicates of N10 *Gar. tubifera* plants located in the row closer to the fence. Scale bar = 21.5cm.

Plate 13. The 4 replicates of control *Hop. ferrea* plants (A) and the 4 replicates of N10 *Hop. ferrea* plants (B). Scale bar = 14cm.

Plate 14. The 5 replicates of control *Lit. sundaicus* plants located in the row further from the fence and the 5 replicates of N10 *Lit. sundaicus* plants located in the row closer to the fence. Scale bar = 21.5cm.

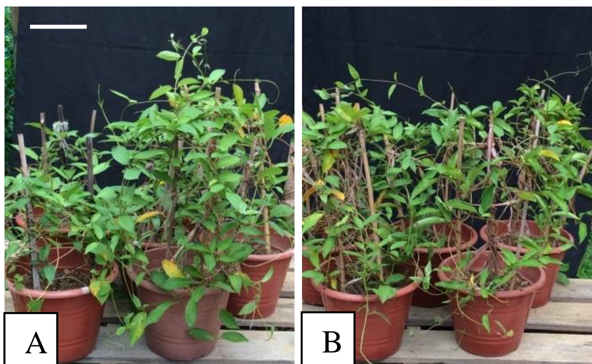

Plate 15. The 5 replicates of control *Pae. foetida* plants (A) and the 5 replicates of N10 *Pae. foetida* plants (B). Scale bar = 14cm.

Plate 16. The 5 replicates of control *Pip. sarmentosum* plants (A) and the 5 replicates of N10 *Pip. sarmentosum* plants (B). Scale bar = 14cm.

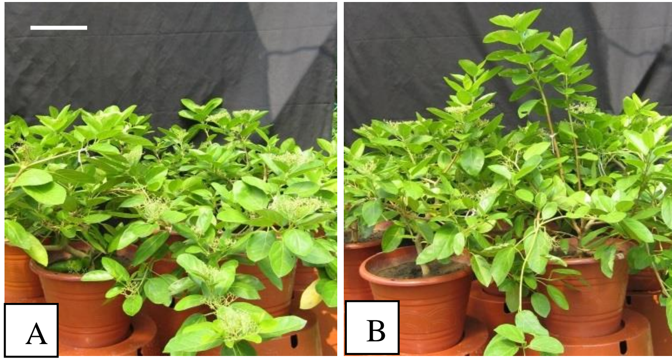

Plate 17. The 5 replicates of control *Pre. serratifolia* plants (A) and the 5 replicates of N10 *Pre. serratifolia* plants (B). Scale bar = 14cm.

Plate 18. The 5 replicates of control *Sch. elliptica* plants located in the row further from the fence and the 5 replicates of N10 *Sch. elliptica* plants located in the row closer to the fence. Scale bar = 14cm.

Plate 19. The 5 replicates of control *Pla. obovata* plants (A) and the 5 replicates of N10 *Pla. obovata* plants (B). Scale bar = 14cm.

Plate 20. The 5 replicates of control *Ste. macrophylla* plants (A) and the 5 replicates of N10 *Ste. macrophylla* plants (B). Scale bar = 14cm.

Plate 21. The 5 replicates of control *Syz. leucoxylon* plants (A) and the 4 replicates of N10 *Syz. leucoxylon* plants (B). Scale bar = 14cm.

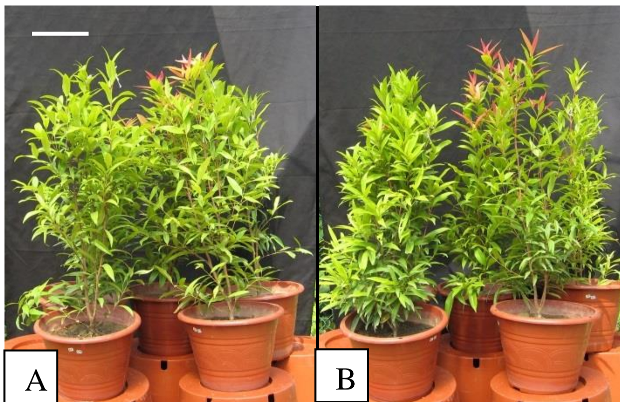

Plate 22. The 5 replicates of control *Syz. myrtifolium* plants (A) and the 5 replicates of N10 *Syz. myrtifolium* plants (B). Scale bar = 14cm.

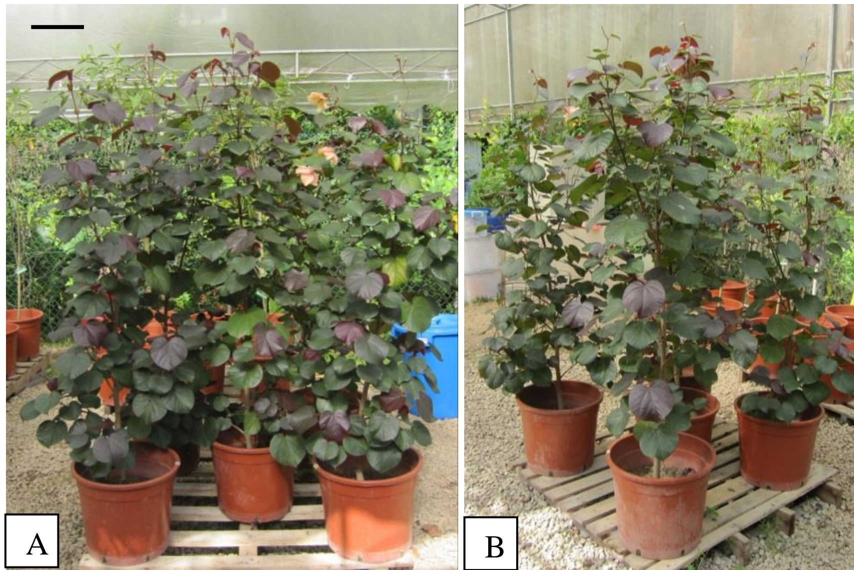

Plate 23. The 5 replicates of control *Tal. tiliaceum* plants (A) and the 5 replicates of N10 *Tal. tiliaceum* plants (B). Scale bar = 21.5cm.

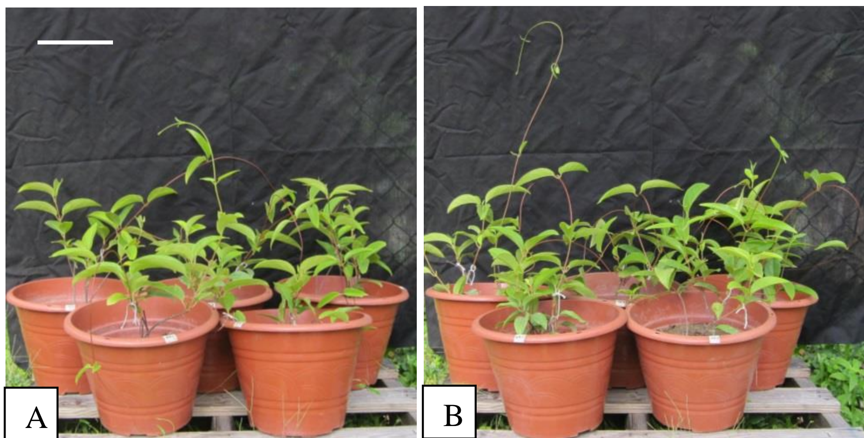

Plate 24. The 5 replicates of control *Tristel. australasiae* plants (A) and the 5 replicates of N10 *Tristel. australasiae* plants (B). Scale bar = 14cm.

Plate 25. The 5 replicates of control *Tar. odorata* plants (A) and the 5 replicates of N10 *Tar. odorata* plants (B). Scale bar = 21.5cm.

Plate 26. The 5 replicates of control *Tristan. whiteana* plants (A) and the 5 replicates of N10 *Tristan. whiteana* plants (B). Scale bar = 21.5cm.

4.2 Chlorophyll fluorescence

The F_v/F_m value refers to the maximum quantum yield of photosystem 2 (PSII) in the dark adapted state. The F_v/F_m values were determined using the Teaching PAM-210 Chlorophyll Fluorometer for *Bar. asiatica*, *Cal. longifolia*, *Cle. sumatranus*, *Cri. asiaticum*, *Ela. tapos*, *Gar. tubifera*, *Lit. sundaicus*, *Pae. foetida*, *Pla. obovata*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, and *Tristan. whiteana*. The F_v/F_m values were determined using the FMS2 for *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Dip. kerrii*, *Hop. ferrea*, *Pip. sarmentosum*, *Pre. serratifolia*, *Sch. elliptica*, *Tar. odorata*, and *Tristel. australasiae*.

Generally, all species maintained F_v/F_m reading in the range of 0.75 – 0.85 (Figures 2 – 5), the typical range for healthy plants (Björkman and Demmig, 1987; Maxwell and Johnson, 2000). The F_v/F_m readings of *Bhe. paniculata* N10 plants were significantly lower than control plants from week 3 – 7 (Figure 2C). Although the F_v/F_m readings for *Che. speciosus* (Figure 2H) and *Gar. tubifera* (Figure 3C) plants were significantly higher at week 1 compared to week 7, these plants still maintained F_v/F_m readings in the healthy range. Lastly, N10 *Syz. myrtifolium* plants and control *Syz. myrtifolium* plants showed F_v/F_m readings below 0.75 during week 5 and week 7 respectively (Figure 4D).

During the non-destructive monitoring period, one plant from each of the following groups had died after repotting: control *Bhe. paniculata*, control and N10 *Hop. ferrea*, and N10 *Syz. leucoxylon*. Thus some species had only 4 replicates while some had 5.

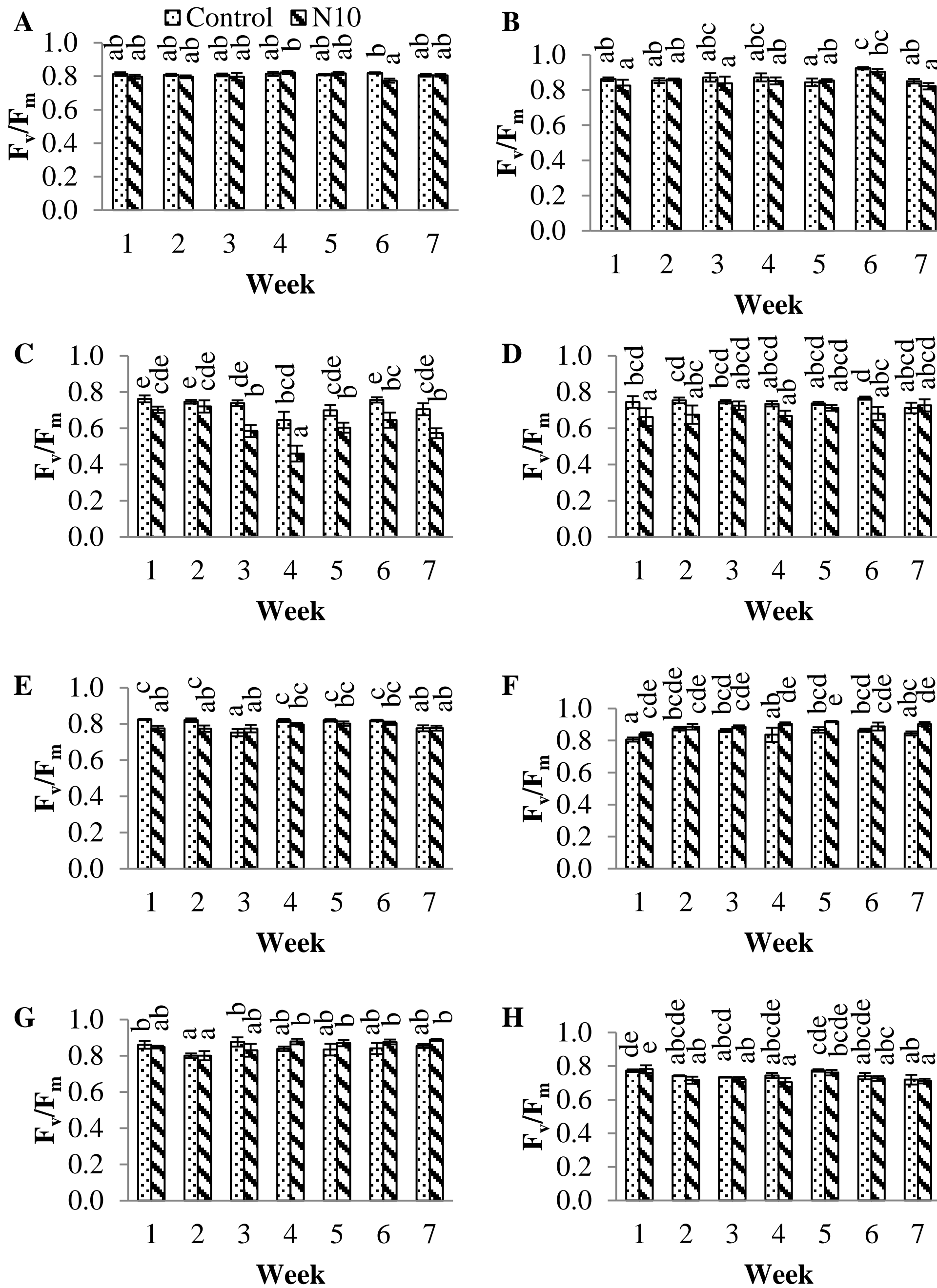

Figure 2. The F_v/F_m values of *Bac. minor* (A), *Bar. asiatica* (B), *Bhe. paniculata* (C), *Bhe. robusta* (D), *Cri. asiaticum* (E), *Cal. longifolia* (F), *Cle. sumatranus* (G), and *Che. speciosus* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

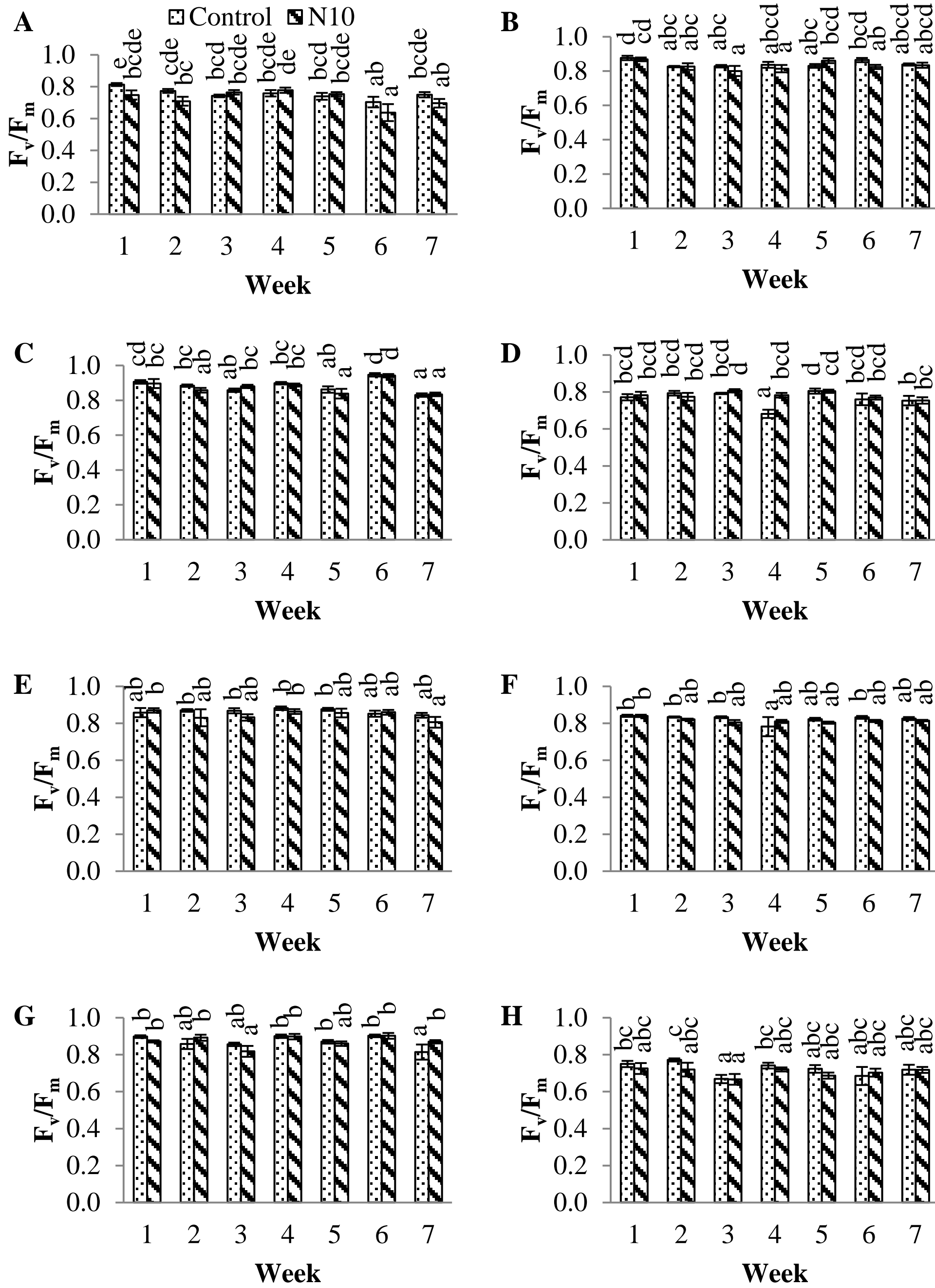

Figure 3. The F_v/F_m values of *Dip. kerrii* (A), *Ela. tapos* (B), *Gar. tubifera* (C), *Hop. ferrea* (D), *Lit. sundaicus* (E), *Pae. foetida* (F), *Pla. obovata* (G), and *Pip. sarmentosum* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

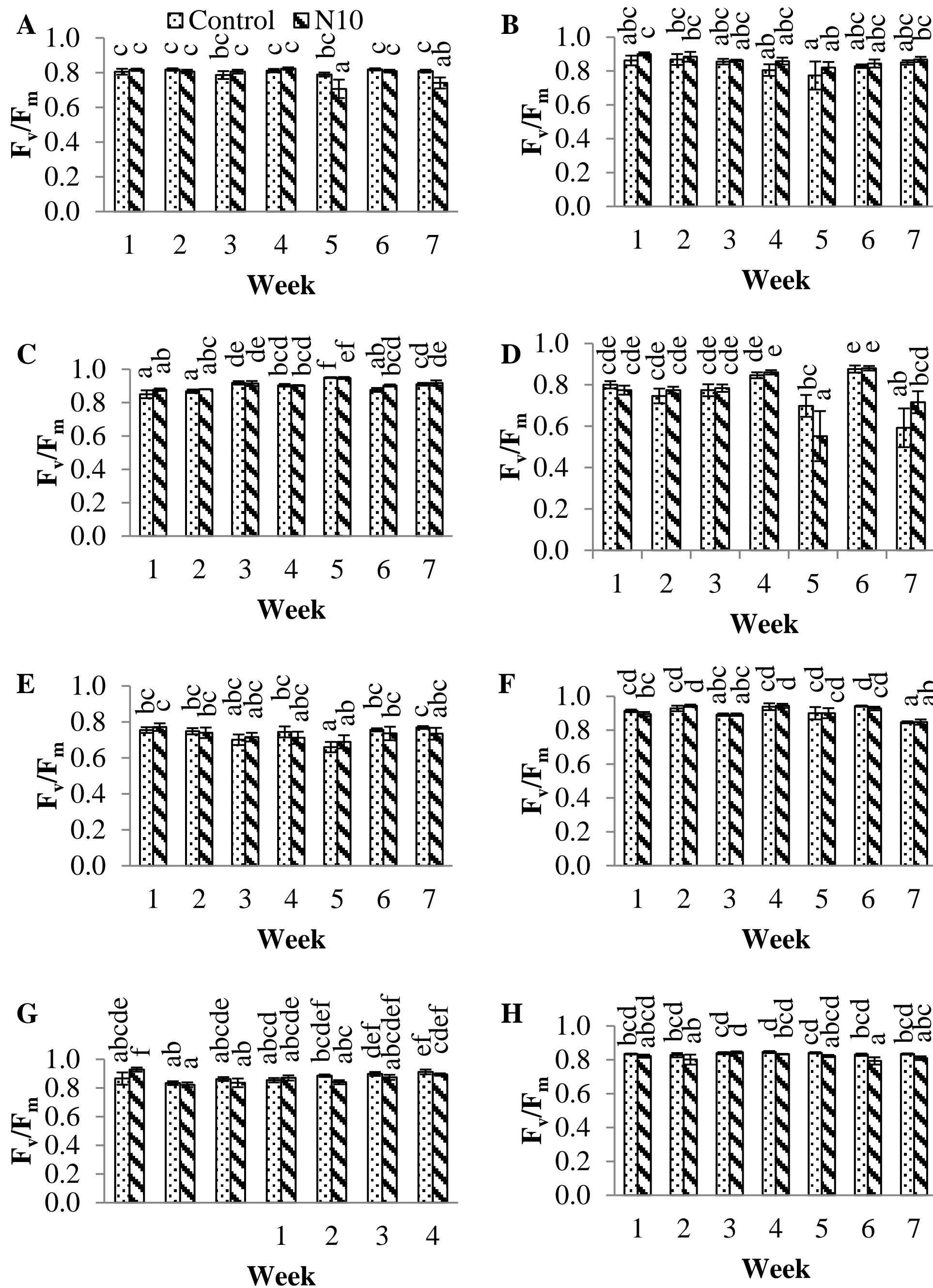

Figure 4. The F_v/F_m values of *Pre. serratifolia* (A), *Ste. macrophylla* (B), *Syz. leucoxylon* (C), *Syz. myrtifolium* (D), *Sch. elliptica* (E), *Tal. tiliaceum* (F), *Tristan. whiteana* (G), and *Tar. odorata* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

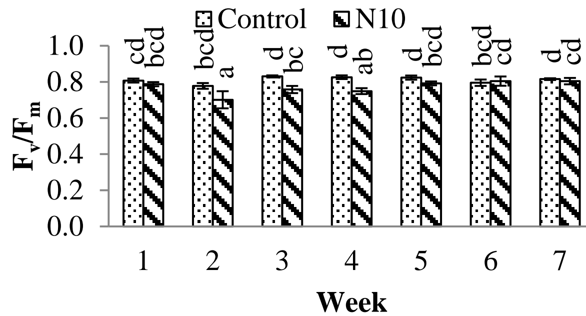

Figure 5. The F_v/F_m values of *Tristel. australasiae* over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

F_v'/F_m' refers to the antennae efficiency of PSII. The F_v'/F_m' readings were determined for *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Dip. kerrii*, *Hop. ferrea*, *Pip. sarmentosum*, *Pre. serratifolia*, *Sch. elliptica*, *Tar. odorata*, and *Tristel. australasiae*. The F_v'/F_m' readings were not significantly different between control and N10 plants of all species throughout the 7 weeks (Figure 6 and 7), except for *Bhe. paniculata* where N10 plants showed significantly lower F_v'/F_m' readings compared to control plants (Figure 6B) during the treatment weeks (week 4 – 7).

The F_v'/F_m' readings were not significantly different between weeks 1 and 7 for all species except *Dip. kerrii* where the F_v'/F_m' readings were significantly lower than week 1 by week 7 (Figure 6E).

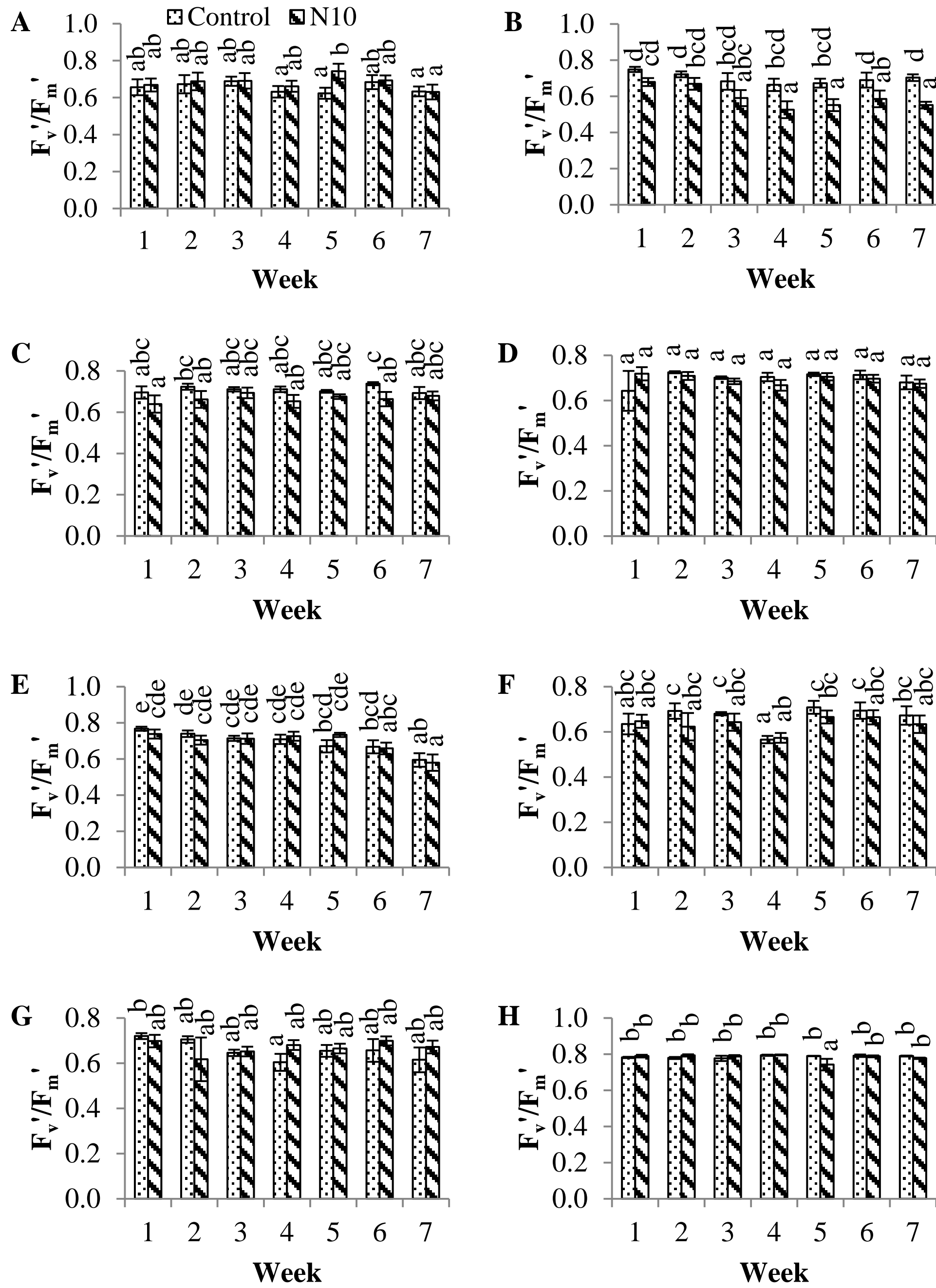

Figure 6. The F_v'/F_m' values for *Bac. minor* (A), *Bhe. paniculata* (B), *Bhe. robusta* (C), *Che. speciosus* (D), *Dip. kerrii* (E), *Hop. ferrea* (F), *Pip. sarmentosum* (G), and *Pre. serratifolia* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for control *Bhe. paniculata* plants and *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

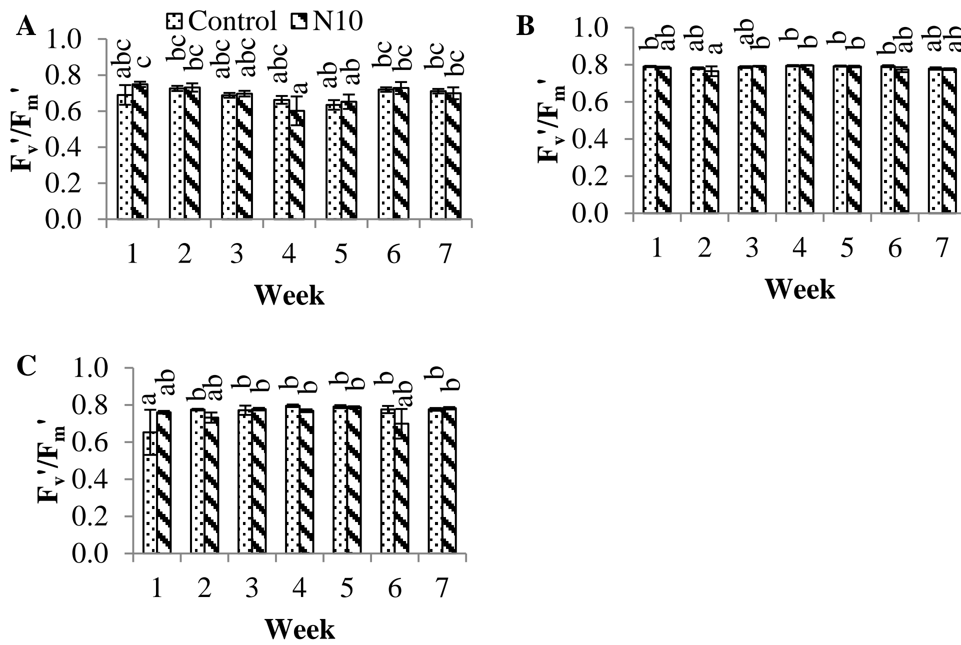

Figure 7. The F_v'/F_m' values for *Sch. elliptica* (A), *Tar. odorata* (B), and *Tristel. australasiae* (C) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

The Φ_{PSII} refers to the quantum efficiency of PSII. The Φ_{PSII} readings were recorded for *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Dip. kerrii*, *Hop. ferrea*, *Pip. sarmentosum*, *Pre. serratifolia*, *Sch. elliptica*, *Tar. odorata*, and *Tristel. australasiae*. The changes in the Φ_{PSII} readings followed the same trend as the F_v'/F_m' readings; the results were not significantly different between control and N10 plants of all species throughout the 7 weeks (Figure 8 and 9), except for *Bhe. paniculata* where N10 plants showed significantly lower Φ_{PSII} readings compared to control plants (Figure 8B) from week 4 – 7.

The Φ_{PSII} readings were also not significantly different between weeks 1 and 7 for all species, following the trend of the F_v'/F_m' readings, except for

Dip. kerrii plants where the F_v'/F_m' readings were significantly lower than week 1 by week 7 (Figure 8E).

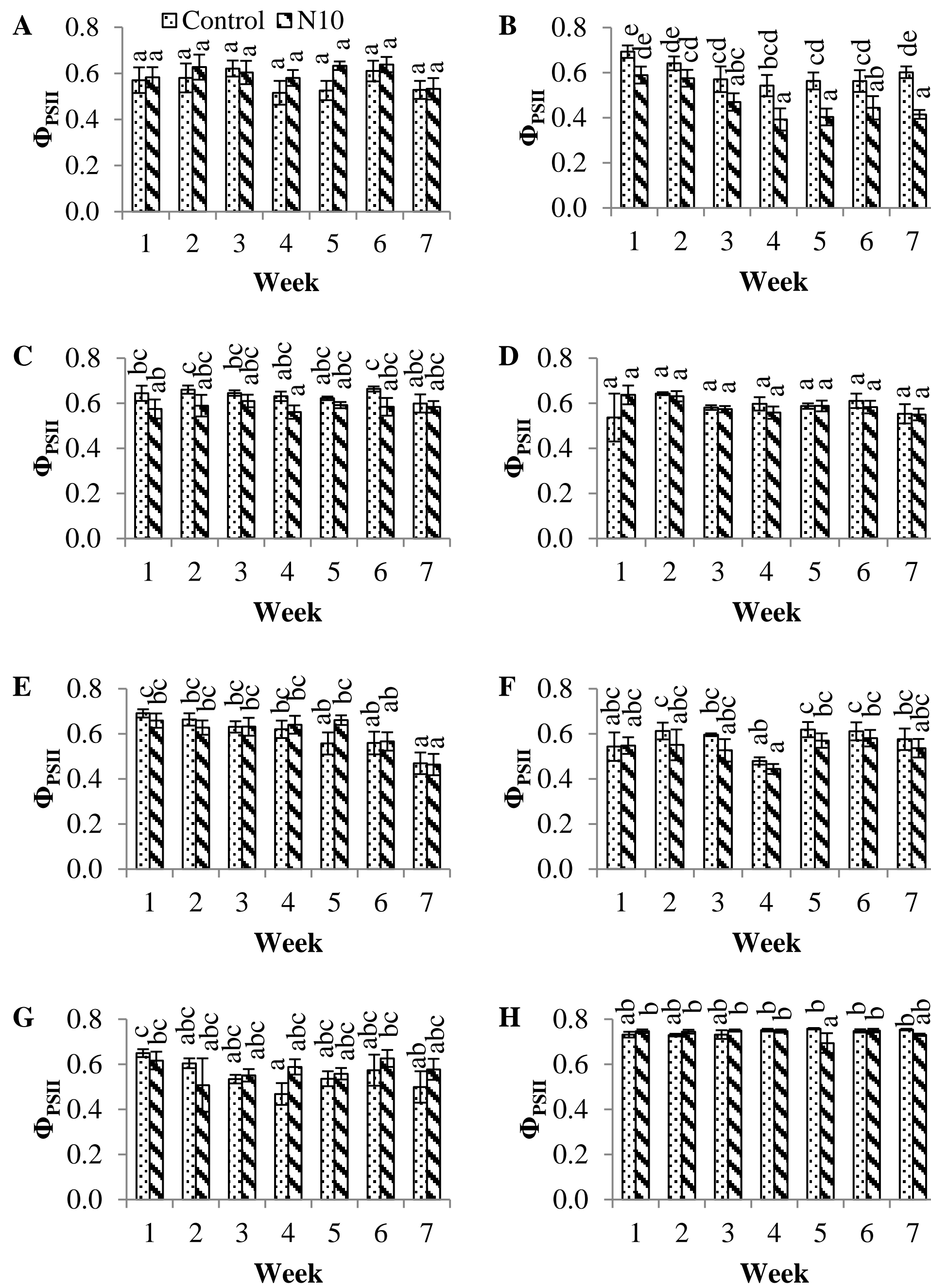

Figure 8. The Φ_{PSII} values for *Bac. minor* (A), *Bhe. paniculata* (B), *Bhe. robusta* (C), *Che. speciosus* (D), *Dip. kerrii* (E), *Hop. ferrea* (F), *Pip. sarmentosum* (G), and *Pre. serratifolia* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for control *Bhe. paniculata* plants and *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

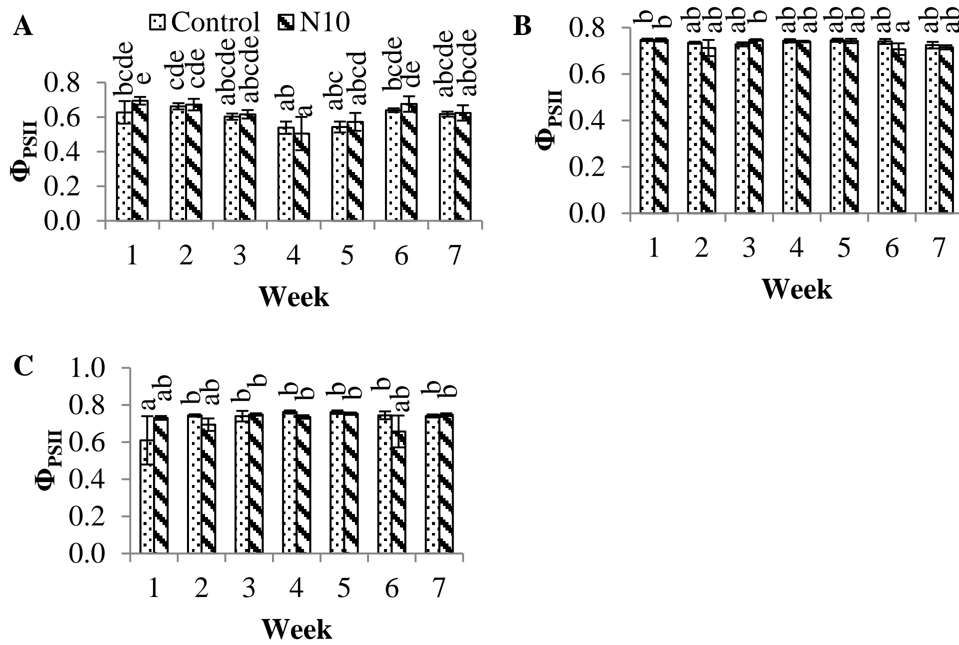

Figure 9. The Φ_{PSII} values for *Sch. elliptica* (A), *Tar. odorata* (B), and *Tristel. australasiae* (C) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

The qP values refers to the photochemical quenching co-efficient and were determined for *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Dip. kerrii*, *Hop. ferrea*, *Pip. sarmentosum*, *Pre. serratifolia*, *Sch. elliptica*, *Tar. odorata*, and *Tristel. australasiae*. The qP values followed a similar trend to the Φ_{PSII} readings where the results were not significantly different between control and N10 plants of all species throughout the 7 weeks (Figure 10 and 11), except for *Bhe. paniculata* where N10 plants showed significantly lower qP values compared to control plants (Figure 10B) during week 5 and 7.

The qP readings were also not significantly different between weeks 1 and 7 for all species, except for *Bhe. robusta* and *Dip. kerrii* plants where the

F_v'/F_m' readings were significantly lower than week 1 by week 7 (Figure 10C and E).

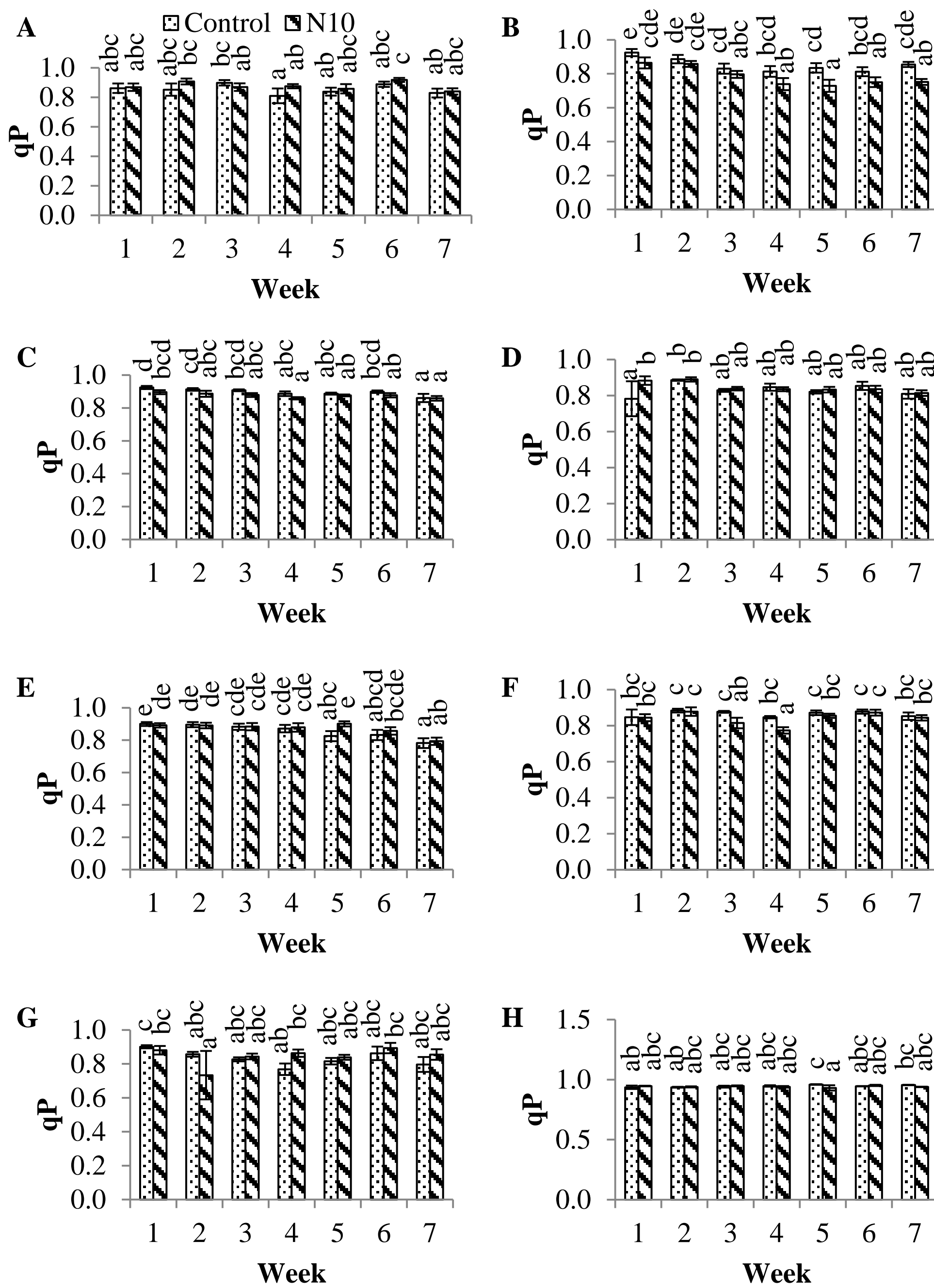

Figure 10. The qP values for *Bac. minor* (A), *Bhe. paniculata* (B), *Bhe. robusta* (C), *Che. speciosus* (D), *Dip. kerrii* (E), *Hop. ferrea* (F), *Pip. sarmentosum* (G), and *Pre. serratifolia* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for control *Bhe. paniculata* plants and *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

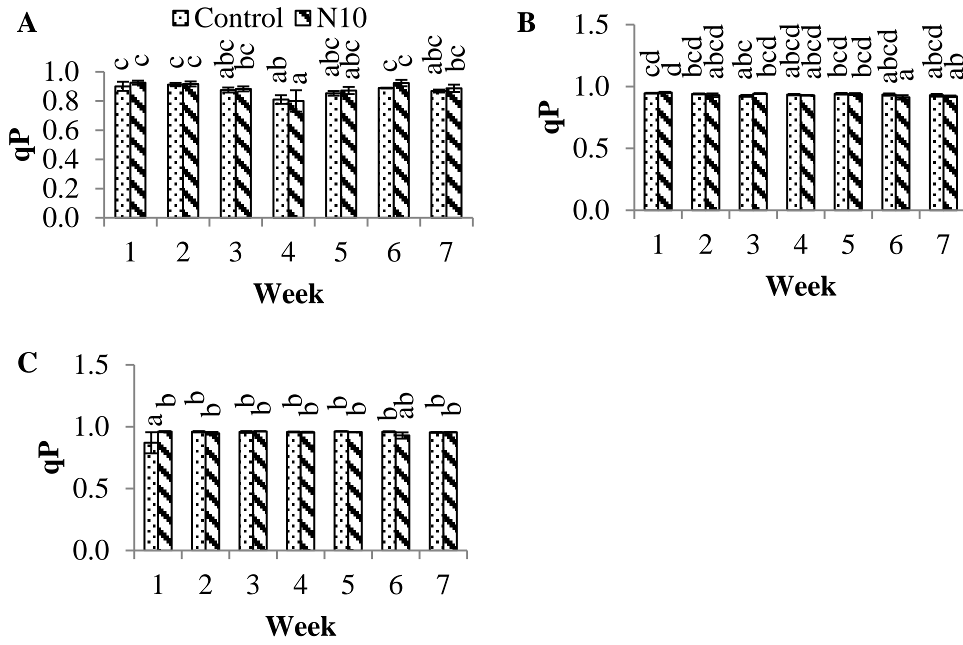

Figure 11. The qP values for *Sch. elliptica* (A), *Tar. odorata* (B), and *Tristel. australasiae* (C) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

The NPQ readings are alternative definition of non-photochemical quenching, and the readings were determined for *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Dip. kerrii*, *Hop. ferrea*, *Pip. sarmentosum*, *Pre. serratifolia*, *Sch. elliptica*, *Tar. odorata*, and *Tristel. australasiae*. There was no significant difference between control and N10 plants for *Bac. minor*, *Bhe. robusta*, *Hop. ferrea*, *Pip. sarmentosum*, *Pre. serratifolia*, *Sch. elliptica*, and *Tar. odorata* (Figure 12A, C, F, G, H and Figure 13A, B). These species also maintained the NPQ readings from week 1 – 7.

Bhe. paniculata N10 plants showed significantly higher NPQ readings compared to the control plants by week 7 (Figure 12B). Although N10 and control plants were not significantly different, *Che. speciosus* plants showed lower NPQ readings at week 7 compared to week 1 (Figure 12D). Both control and N10 *Dip. kerrii* plants showed significantly higher NPQ readings at week 7 compared to week 1 (Figure 12E). And *Tristel. australasiae* control plants showed in signicantly higher NPQ reading compared to N10 plants only during week 3 (Figure 13C).

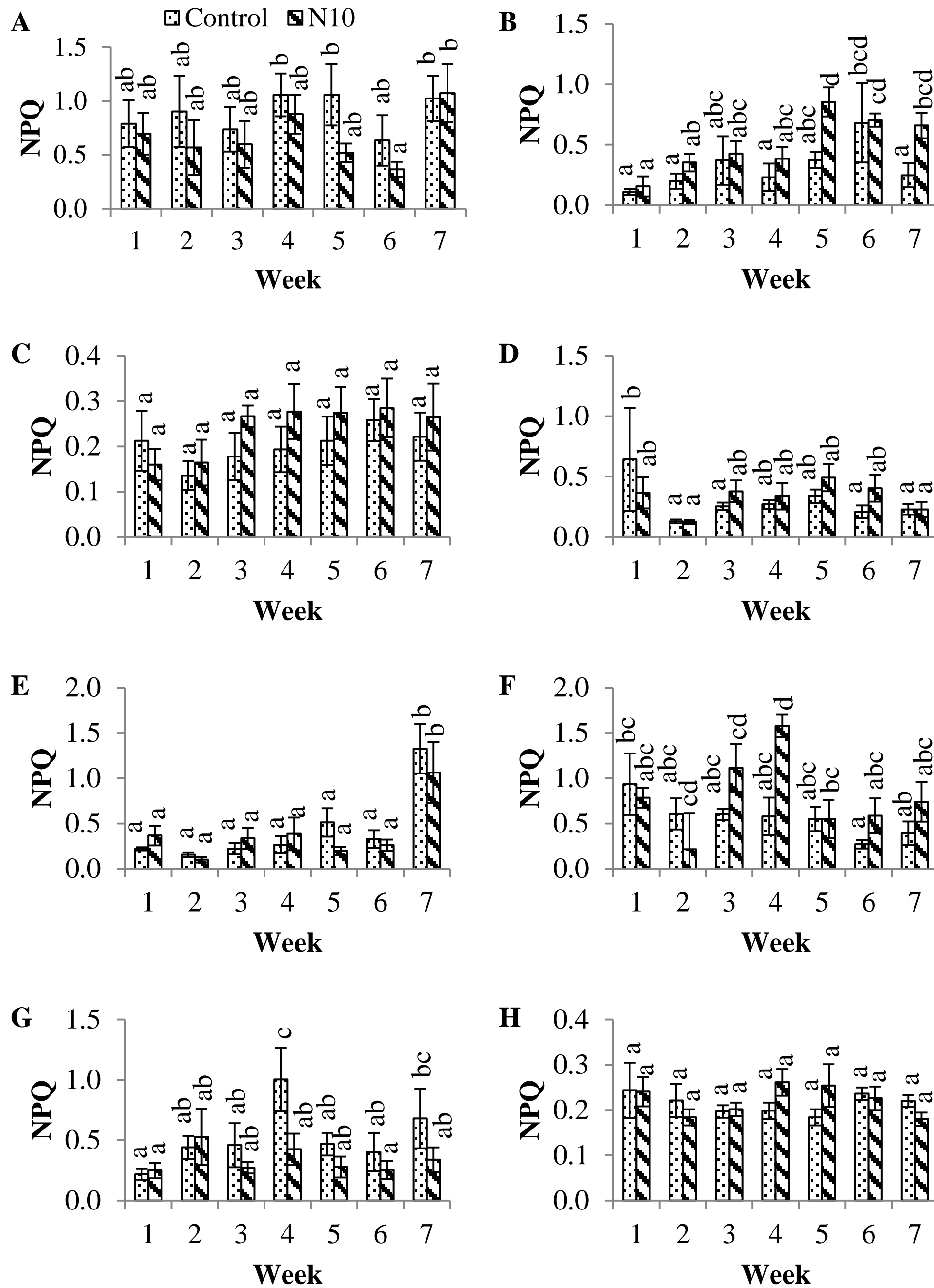

Figure 12. The NPQ values for *Bac. minor* (A), *Bhe. paniculata* (B), *Bhe. robusta* (C), *Che. speciosus* (D), *Dip. kerrii* (E), *Hop. ferrea* (F), *Pip. sarmentosum* (G), and *Pre. serratifolia* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for control *Bhe. paniculata* plants and *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

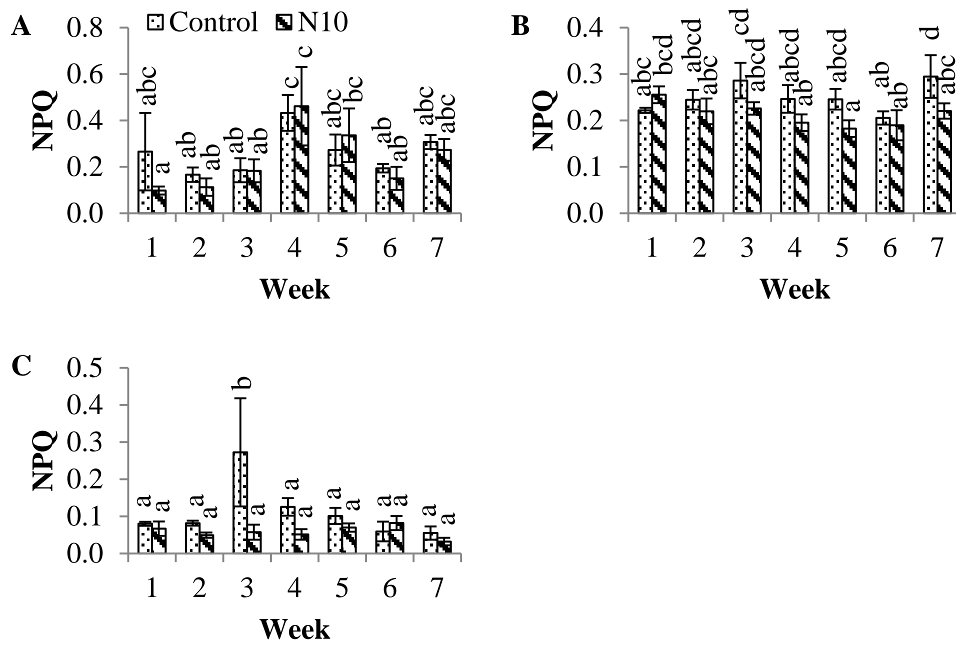

Figure 13. The NPQ values for *Sch. elliptica* (A), *Tar. odorata* (B), and *Tristel. australasiae* (C) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

4.3 SPAD

The SPAD readings for *Bac. minor* (Figure 14A), *Bar. asiatica* (Figure 14B), *Bhe. paniculata* (Figure 14C), *Cle. sumatranus* (Figure 14G), *Dip. kerrii* (Figure 15A), *Ela. tapos* (Figure 15B), *Hop. ferrea* (Figure 15D), *Pae. foetida* (Figure 15F), *Pla. obovata* (Figure 15G), *Sch. elliptica* (Figure 16E), *Ste. macrophylla* (Figure 16B), *Syz. leucoxylon* (Figure 16C), *Syz. myrtifolium* (Figure 16D), *Tal. tiliaceum* (Figure 16F), *Tar. odorata* (Figure 16H), and *Tristan. whiteana* (Figure 16G) were not significantly different between control and N10 groups, as well as from week 1 – 7.

The SPAD readings for *Bhe. robusta* (Figure 14D), *Gar. tubifera* (Figure 15C), *Lit. sundaicus* (Figure 15E), and *Tristel. australasiae* (Figure 17) control plants decreased significantly from week 1 to week 7, but those of N10 plants remained consistent throughout all weeks.

Both control and N10 plants of *Cri. asiaticum* (Figure 14E), *Cal. longifolia* (Figure 14F), *Che. speciosus* (Figure 14H), *Pip. sarmentosum* (Figure 15H), and *Pre. serratifolia* (Figure 16A) showed decreasing SPAD readings from week 1 to 7.

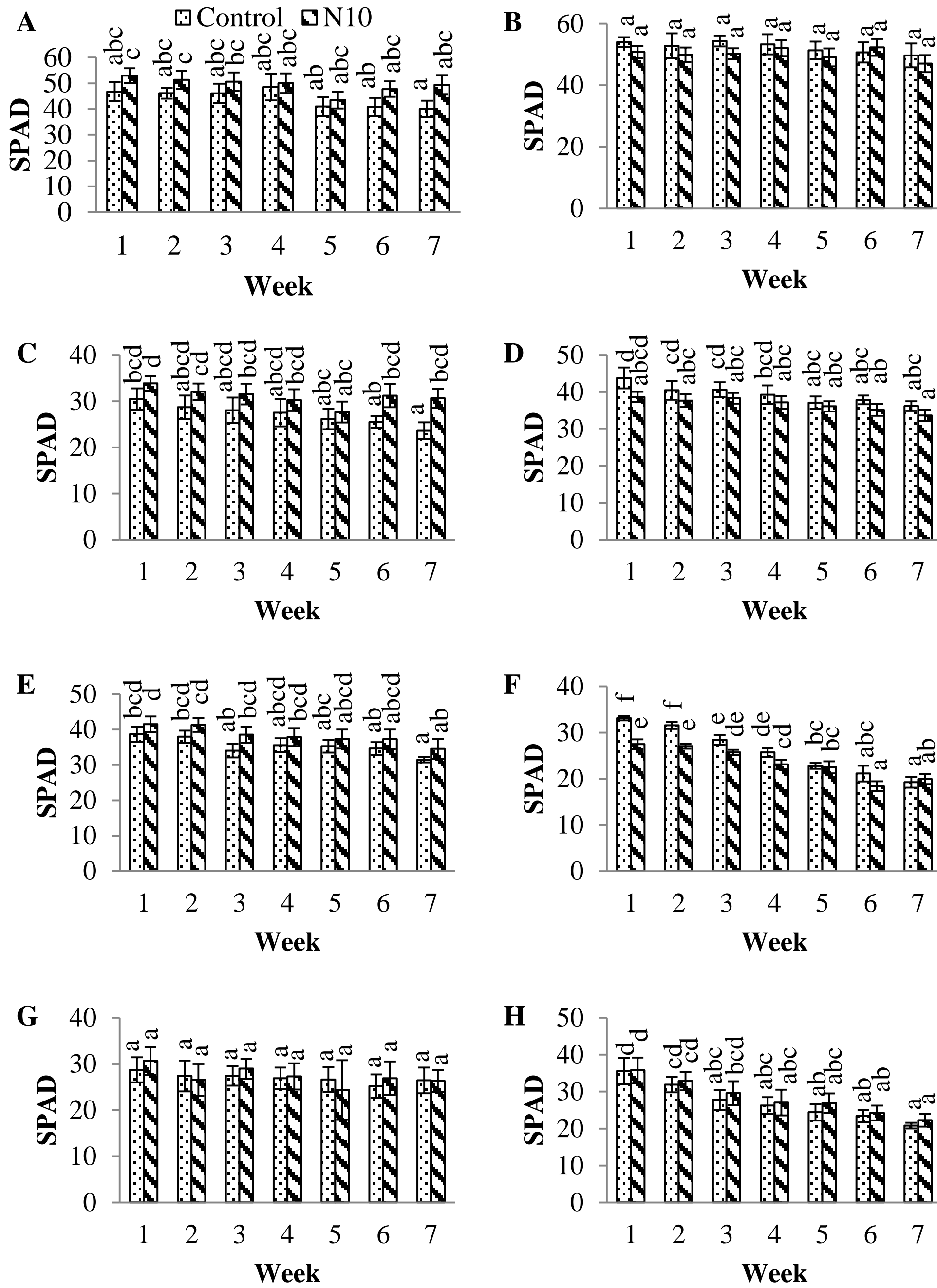

Figure 14. The SPAD values of *Bac. minor* (A), *Bar. asiatica* (B), *Bhe. paniculata* (C), *Bhe. robusta* (D), *Cri. asiaticum* (E), *Cal. longifolia* (F), *Cle. sumatranus* (G), and *Che. speciosus* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

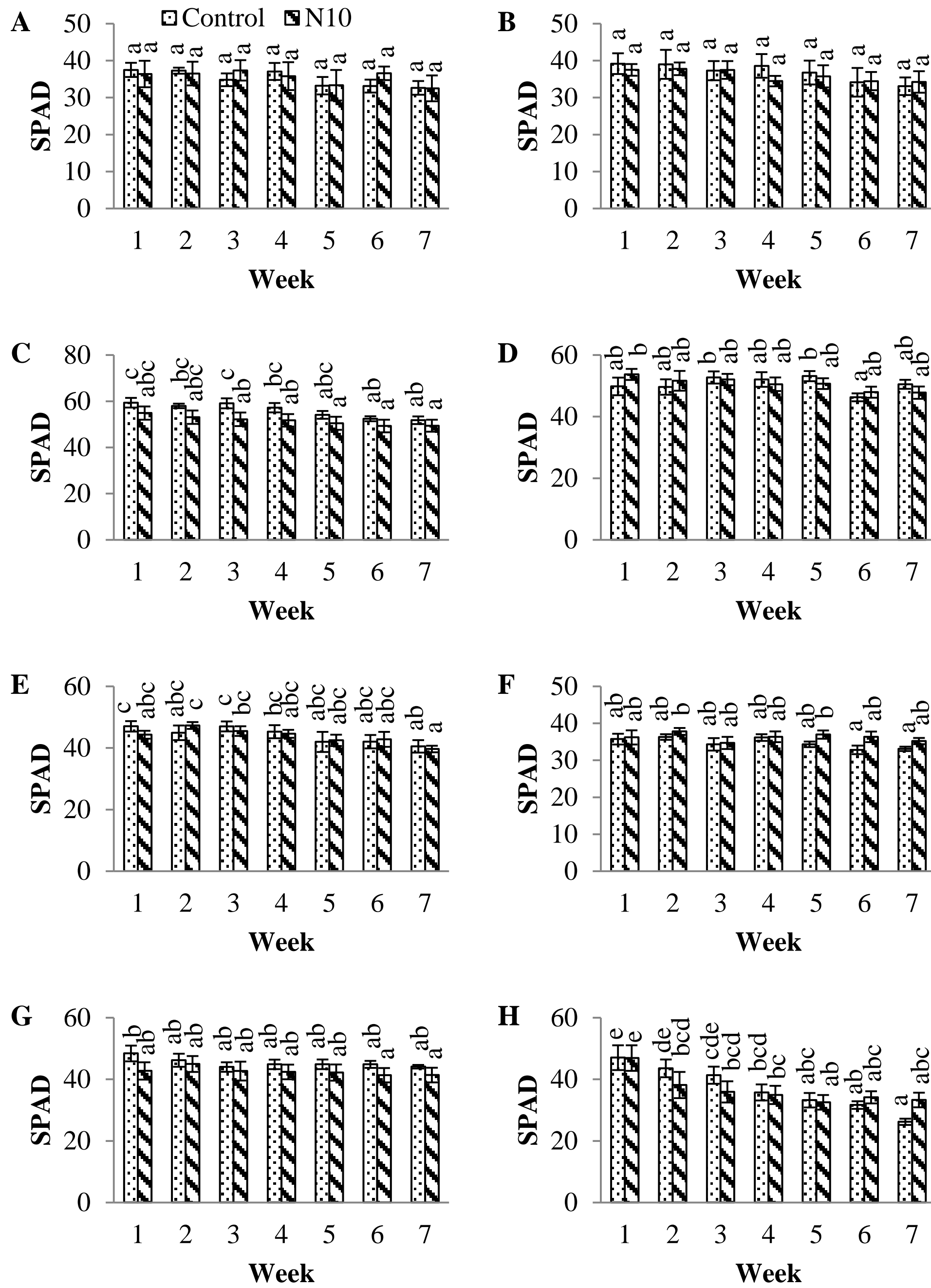

Figure 15. The SPAD values of *Dip. kerrii* (A), *Ela. tapos* (B), *Gar. tubifera* (C), *Hop. ferrea* (D), *Lit. sundaicus* (E), *Pae. foetida* (F), *Pla. obovata* (G), and *Pip. sarmentosum* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher’s least significant difference (LSD) test.

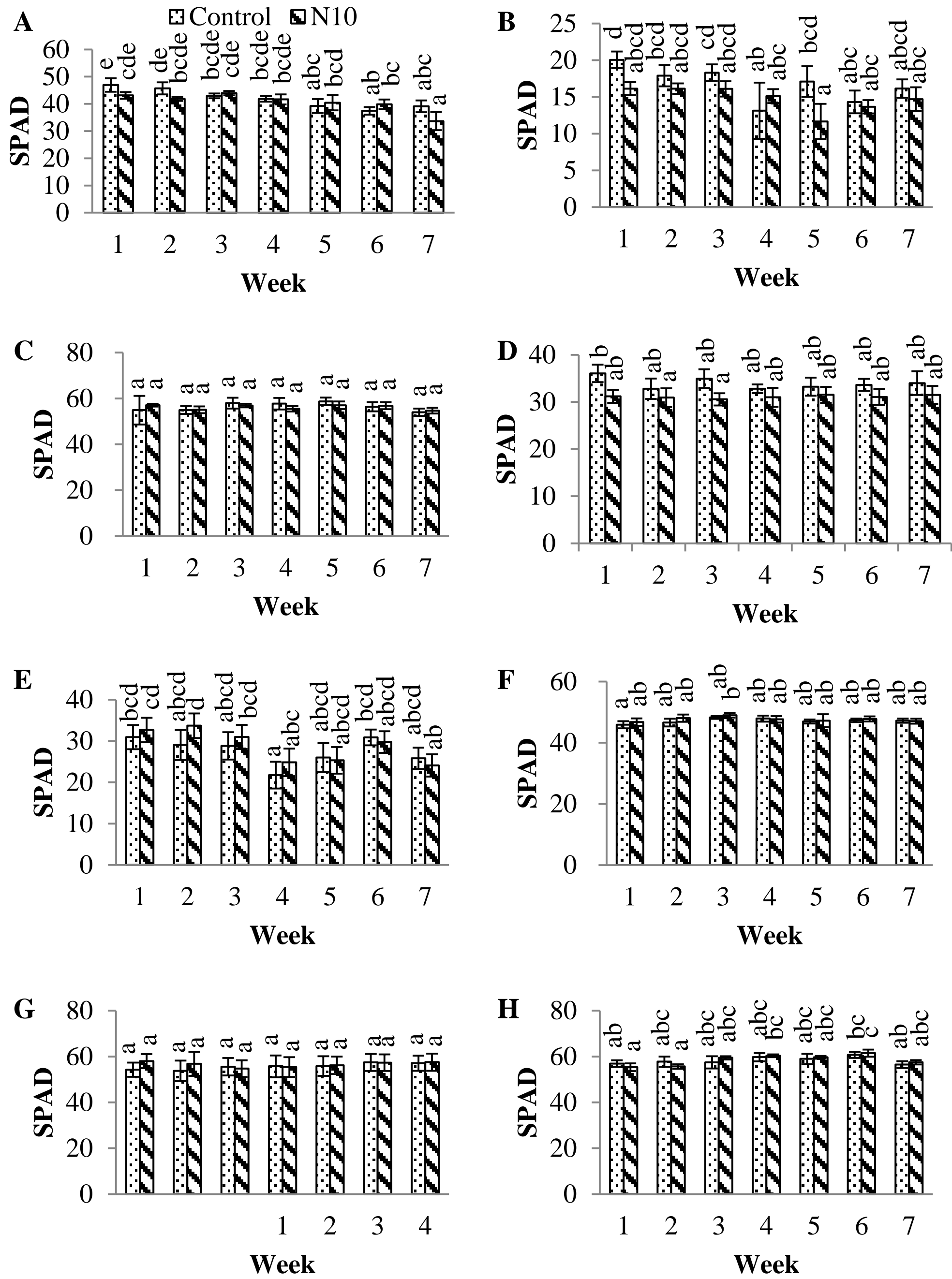

Figure 16. The SPAD values of *Pre. serratifolia* (A), *Ste. macrophylla* (B), *Syz. leucoxylon* (C), *Syz. myrtifolium* (D), *Sch. elliptica* (E), *Tal. tiliaceum* (F), *Tristan. whiteana* (G), and *Tar. odorata* (H) over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

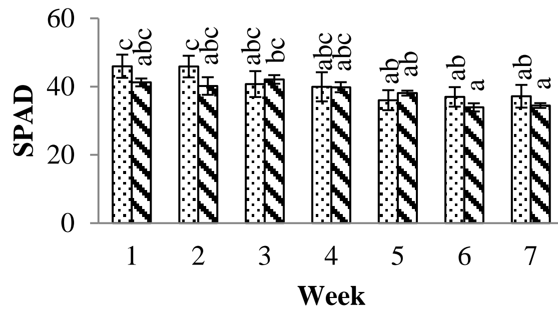

Figure 17. The SPAD values of *Tristel. australasiae* over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

4.4 Leaf length

The leaf length of *Bac. minor*, *Bar. asiatica*, *Bhe. paniculata*, *Bhe. robusta*, *Cri. asiaticum*, *Che. speciosus*, *Cle. sumatranus*, *Ela. tapos*, *Gar. tubifera*, *Hop. ferrea*, *Lit. sundaicus*, *Pae. foetida*, *Pip. sarmentosum*, *Pla. obovata*, *Pre. serratifolia*, *Sch. elliptica*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tristan. whiteana*, and *Tristel. australasiae* plants did not differ significantly between control and N10 plants, as well as between weeks 1, 4, and 7 (Figure 18). Mature leaf length remained constant over the weeks, with insignificant fluctuations.

The leaf length of *Cal. longifolia* N10 plants was significantly shorter than the leaf length of control plants (Figure 18). *Dip. kerrii* control plants also showed significantly longer leaves than N10 plants except for the last week, when the difference was no longer significant (Figure 18). Lastly, *Tar. odorata* N10 and control plants both showed significantly smaller leaves at week 4 and 7 compared to week 1 (Figure 18).

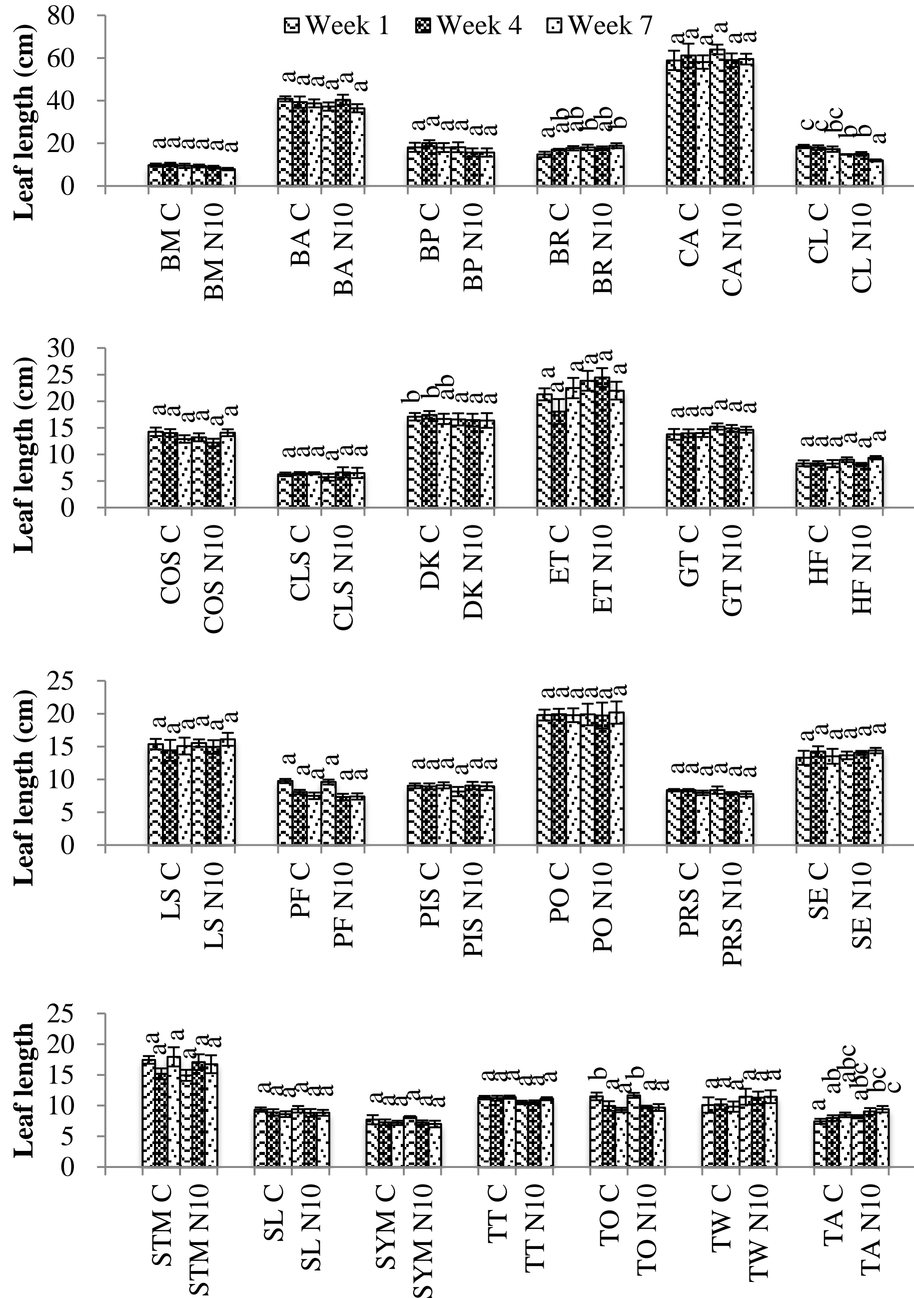

Figure 18. The leaf length of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants recorded on week 1, 4, and 7. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 within the same species.

4.5 Total number of leaves and the number of leaves on a growing branch

The total number of leaves was counted for plants which had less than 150 leaves in total. The total number of leaves of *Bar. asiatica*, *Bhe. paniculata*, *Bhe. robusta*, *Cal. longifolia*, *Cri. asiaticum*, *Dip. kerrii*, *Ela. tapos*, *Pip. sarmentosum*, *Pla. obovata*, *Sch. elliptica*, *Tristan. whiteana*, and *Tristel. australasiae* control and N10 plants did not differ significantly between week 4 and 7 (Figure 19). All the plants for which the total number of leaves was counted maintained similar number of leaves at the start and end of the treatment period except *Ste. macrophylla* control and N10 plants which showed a significant increase in the total number of leaves from week 4 to 7 (Figure 19).

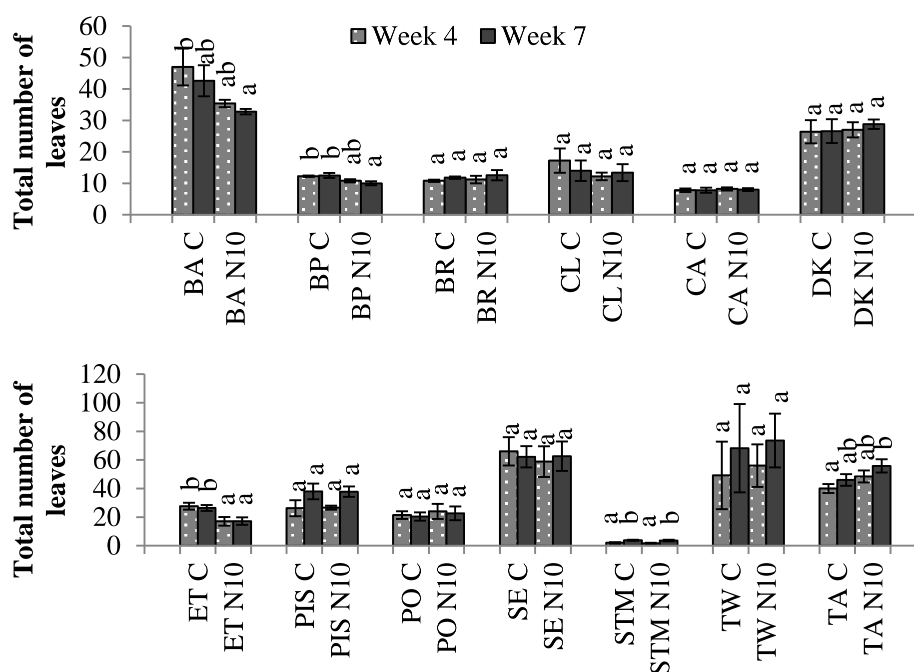

Figure 19. The total number of leaves of *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cal. longifolia* (CL), *Cri. asiaticum* (CA), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants recorded on week 4 and 7. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 within the same species.

The number of new leaves on a growing branch was based on week 1 as a baseline. *Bhe. paniculata* and *Tristan. whiteana* control plants showed significantly more new leaves grown on a growing branch at week 7 compared to week 4, but the same trend was not observed for the N10 plants of these species (Figure 20).

The *Bhe. robusta*, *Pip. sarmentosum*, and *Ste. macrophylla* control and N10 plants both showed significantly more new leaf growth at week 7 compared to week 4 (Figure 20). Although both control and N10 *Syz. leucoxylon* plants showed significantly higher number of new leaves at week 7 compared to week 4; *Syz. leucoxylon* N10 plants also had significantly higher number of new leaves compared to control plants (Figure 20).

Cal. longifolia, *Lit. sundaicus*, and *Sch. elliptica* plants showed significantly more leaves on a growing branch at week 7 compared to week 4. However, the control plants showed no significant difference in the number of new leaves between week 4 and 7 (Figure 20).

Finally, the new leaf growth for *Bac. minor*, *Bar. asiatica*, *Che. speciosus*, *Cri. asiaticum*, *Dip. kerrii*, *Ela. tapos*, *Gar. tubifera*, *Hop. ferrea*, *Pae. foetida*, *Pla. obovata*, *Pre. serratifolia*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, and *Tristel. australasiae* control and N10 plants showed no significant differences in new leaf growth from weeks 4 to 7 (Figure 20).

Cle. sumatranus plants did not produce any new leaves and the plants had too many leaves to count the total number of leaves accurately. Thus this species has no results to be presented in this section.

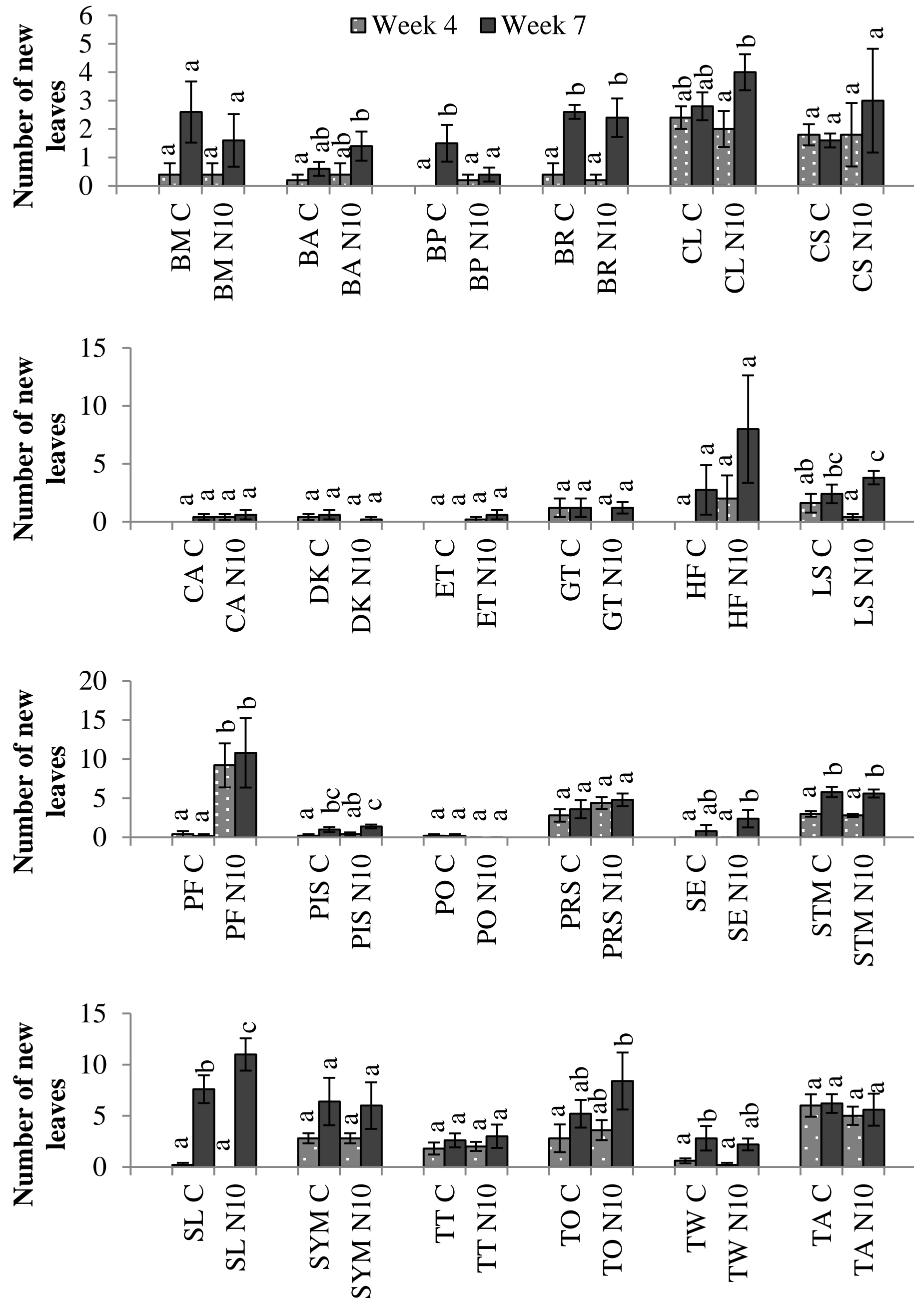

Figure 20. The number of new leaves of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cal. longifolia* (CL), *Che. speciosus* (CS), *Cri. asiaticum* (CA), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants on a growing branch recorded on week 4 and 7. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 within the same species.

4.6 Visual quality

The visual quality of the plants is presented as a mean of the scores from all 7 weeks and shows an overall score (Figure 21). None of the plants scored the full 15, 5 for wilt, 5 for burn, and 5 for yellowing. However, all were very close to the target score of 15 and control and N10 plants of the same species did not differ much.

Wilt accounted for the least number of poor visual quality scores, where only *Bar. asiatica*, *Pre. serratifolia*, and *Syz. myrtifolium* control and N10 plants had wilt as the lowest visual quality score (compared to burn and yellowing) at 4.44, 3.94, and 4.66 respectively (Figure 21). Even though the scores for wilt were the lowest compared to burn and yellowing, the score meant that the plants had only a few leaves which were wilted (Table 2) and the overall visual quality score was still high.

The second visual quality assessment criterion that accounted for the next lowest scores was yellowing, where *Che. speciosus*, *Gar. tubifera*, *Pae. foetida*, *Pip. sarmentosum*, *Sch. elliptica*, *Ste. macrophylla*, and *Tar. odorata* control and N10 plants scored 3.94, 3.99, 4.01, 4.11, 4.49, 4.67, and 3.83 respectively (Figure 21). Although the SPAD readings for the matured leaves of *Pae. foetida* (Figure 15F), *Sch. elliptica* (Figure 16E), *Ste. macrophylla* (Figure 16B), and *Tar. odorata* (Figure 16H) control and N10 plants were not significantly different from week 1 – 7, the plants had a few yellow leaves overall. SPAD values of mature leaves of *Gar. tubifera* (Figure 15C) control plants decreased significantly compared to N10 plants but overall both control and N10 plants had a few yellow leaves. *Che. speciosus* (Figure 14H) and *Pip. sarmentosum* (Figure 15H) control and N10 plants both showed decreasing

SPAD readings from week 1 to 7, and their yellowing scores were close to 4, representing a few yellow leaves (Table 2). However, even though the plants had a few yellow leaves, they were not wilted or burnt and still had a good overall visual quality score.

Burns were the most common cause for the less than perfect visual quality scores, where *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Cal. longifolia*, *Cle. sumatranus*, *Cri. asiaticum*, *Dip. kerrii*, *Ela. tapos*, *Hop. ferrea*, *Lit. sundaicus*, *Pla. obovata*, *Syz. leucoxylon*, *Tal. tiliaceum*, *Tristan. whiteana*, and *Tristel. australasiae* scored 3.97, 4.66, 4.34, 4.16, 4.03, 4.00, 3.99, 4.27, 4.13, 4.00, 4.43, 4.24, 4.41, 4.03, and 4.66 respectively (Figure 21). Even though the burn scores for these plants were the lowest compared to the wilt and yellowing scores, the burns were only seen at the tips or edges of the leaves (Table 2). Overall, the plants were still considered to have high visual quality, close to the perfect score of 15.

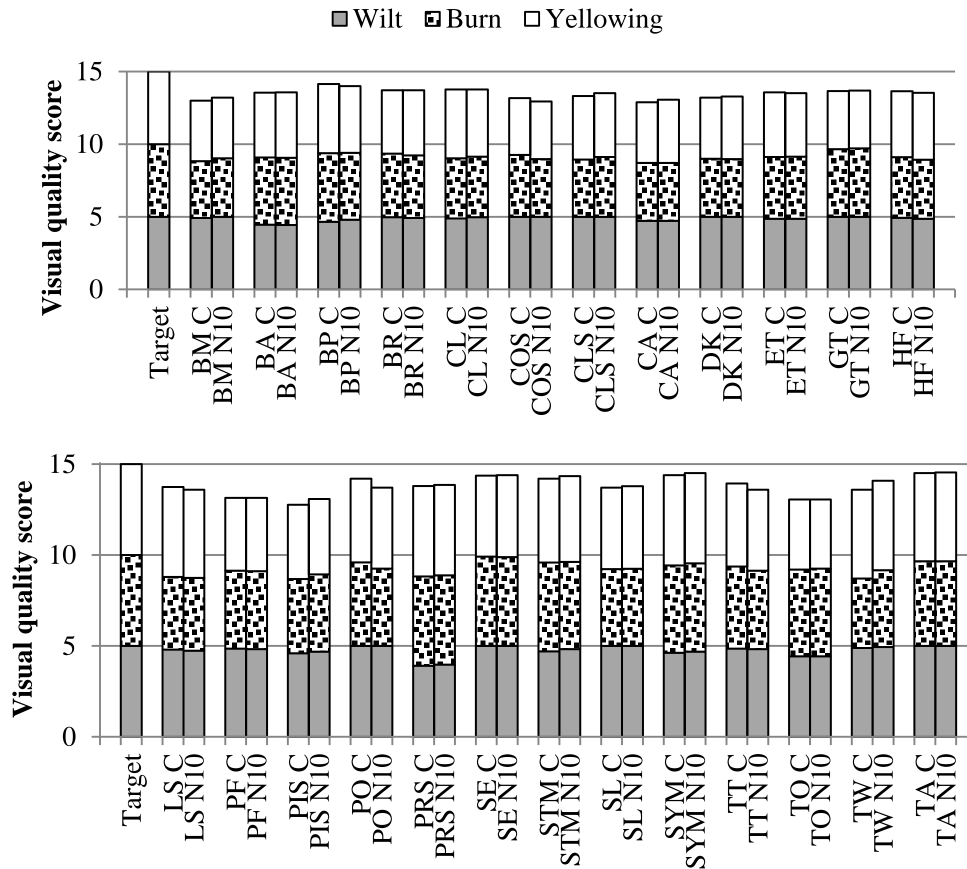

Figure 21. The visual quality scores of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Cri. asiaticum* (CA), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants. Each bar represent the mean of 5 replicates over a period of 7 weeks except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates.

4.7 Soil moisture

In general, for all plant species, the volumetric water content did not differ significantly between control and N10 groups (Figures 22 – 25). Furthermore, pots planted with *Bhe. paniculata*, *Che. speciosus*, *Ela. tapos*, *Hop. ferrea*, *Pip. sarmentosum*, *Pla. obovata*, *Sch. elliptica*, *Ste. macrophylla*, and *Tristan. whiteana* plants and barren pots of soil did not differ significantly (Figures 22C and F, 23D and F, 24A, B, D and E, and 25B).

However, differences were observed between pots with plants and barren pots for some species. Pots planted with *Bhe. robusta*, *Cal. longifolia*, and *Dip. kerrii* plants maintained a relatively similar volumetric water content throughout the 7 weeks, but pots with barren soil increased in water content significantly from week 1 to 7 (Figures 22D and E, and 23C). Pots planted with *Bar. asiatica*, *Cle. sumatranus*, *Cri. asiaticum*, *Gar. tubifera*, *Lit. sundaicus*, *Pae. foetida*, *Pre. serratifolia*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, and *Tar. odorata* plants had a significantly lower water content compared to the pots with barren soil at end of the experiments although they showed little difference at the beginning (Figures 22B, 23A, B, E, G, and H, 24C, F, G, and H, and 25A). This was the most common trend observed, with 11 out of 25 species showing this trend.

Pots with *Tristel. australasiae* plants had significantly lower water content at week 7 compared to beginning of the experiments, whereas the pots with barren soil maintained the water content and was significantly higher than pots with the plants at the week 7 (Figure 25C).

And the only species which showed a significantly higher water content compared to the soil at week 7 was *Bac. minor* (Figure 22A).

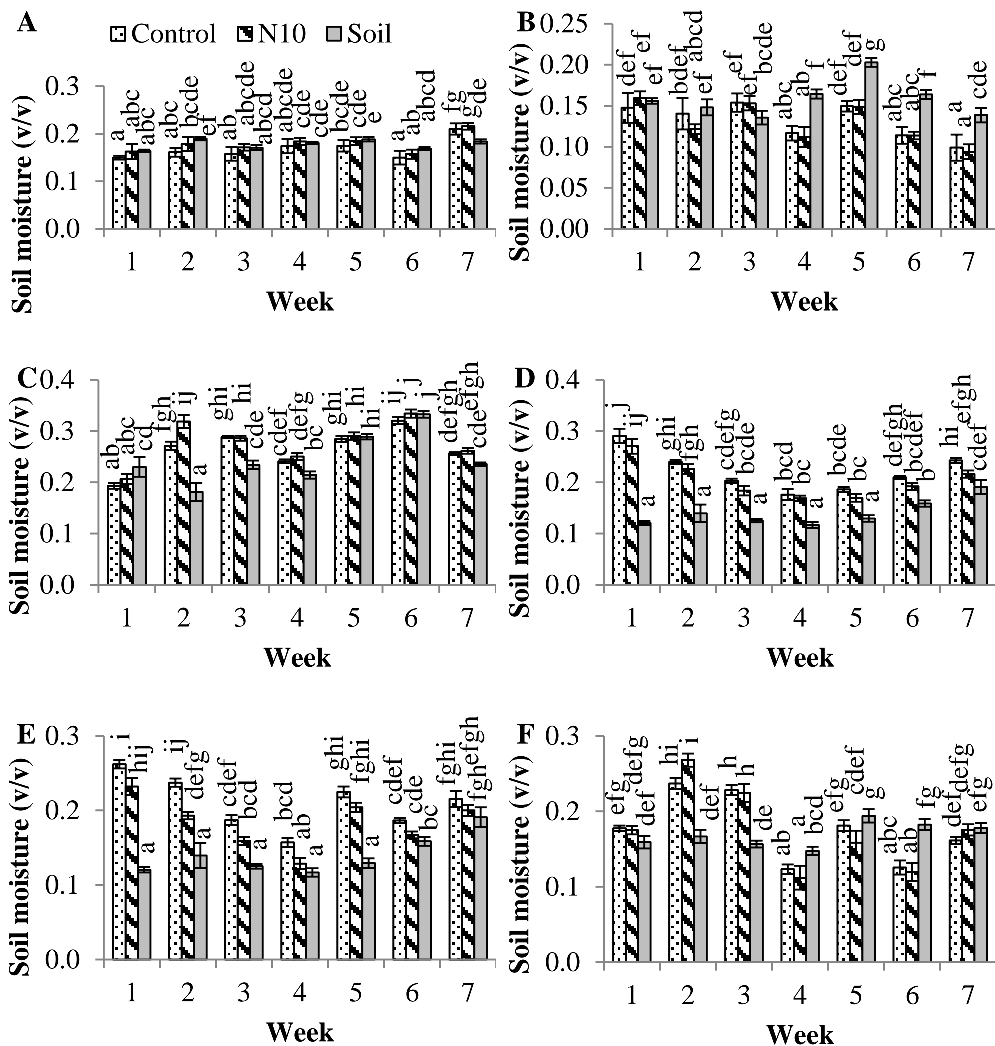

Figure 22. The volumetric water content of the pots with barren soil and pots planted with *Bac. minor* (A), *Bar. asiatica* (B), *Bhe. paniculata* (C), *Bhe. robusta* (D), *Cal. longifolia* (E), and *Che. speciosus* (F) control and N10 plants over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher’s least significant difference (LSD) test.

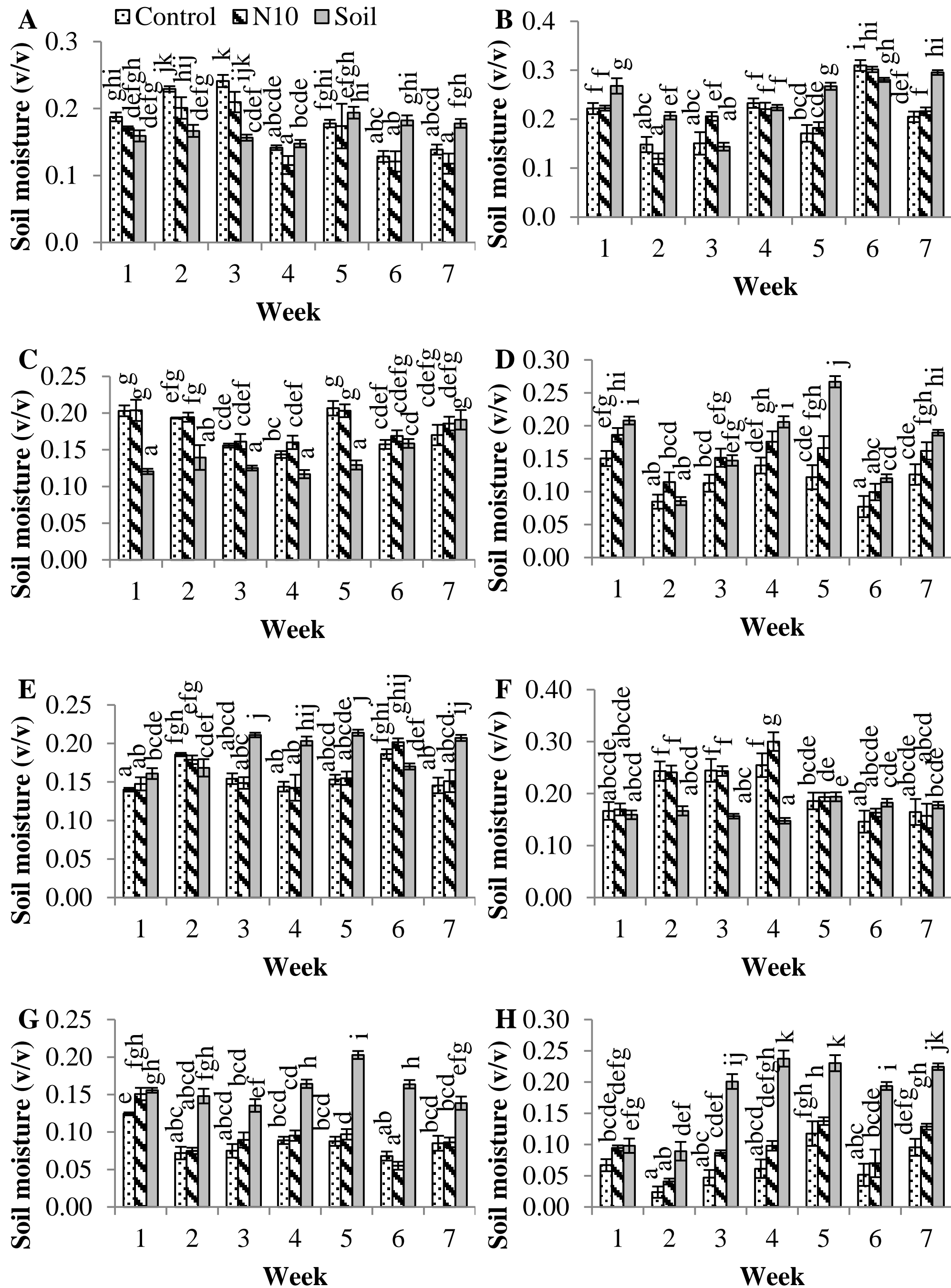

Figure 23. The volumetric water content of the pots with barren soil and pots planted with *Cle. sumatranus* (A), *Cri. asiaticum* (B), *Dip. kerrii* (C), *Ela. tapos* (D), *Gar. tubifera* (E), *Hop. ferrea* (F), *Lit. sundaicus* (G), and *Pae. foetida* (H) control and N10 plants over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

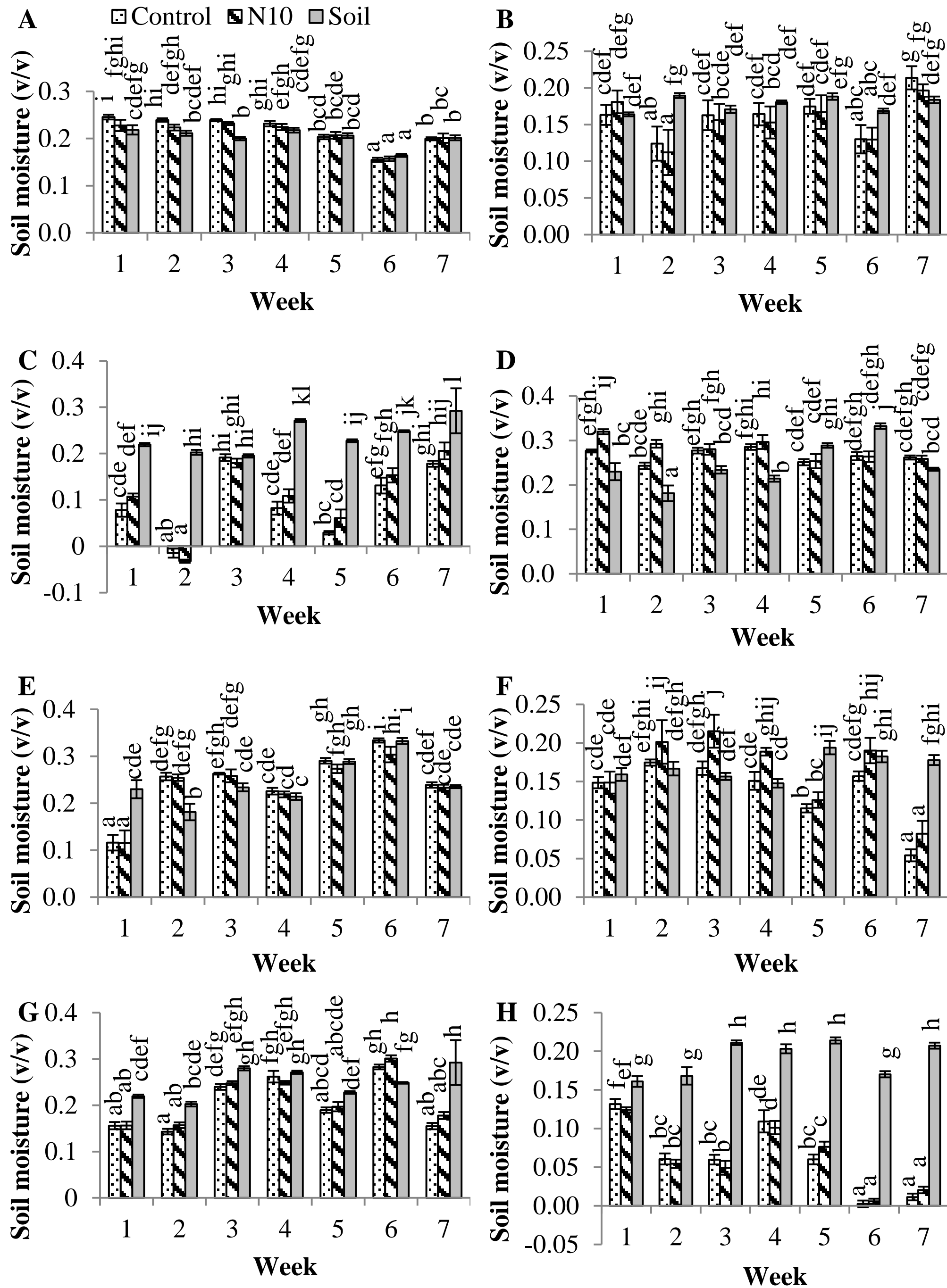

Figure 24. The volumetric water content of the pots with barren soil and pots planted with *Pip. sarmentosum* (A), *Pla. obovata* (B), *Pre. serratifolia* (C), *Sch. elliptica* (D), *Ste. macrophylla* (E), *Syz. leucoxylon* (F), *Syz. myrtifolium* (G), and *Tal. tiliaceum* (H) control and N10 plants over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

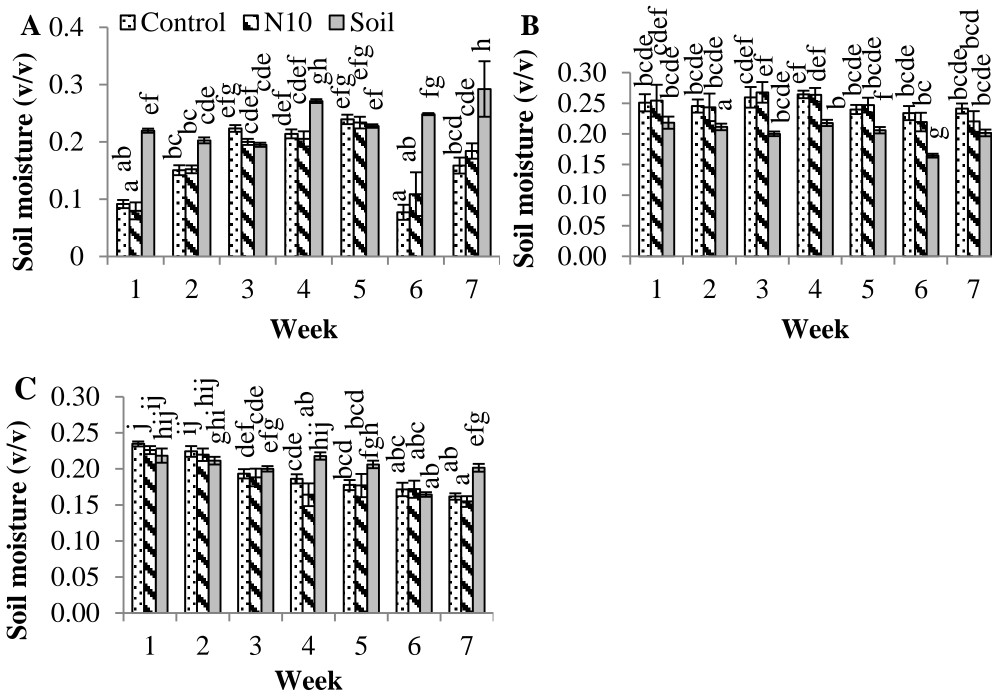

Figure 25. The volumetric water content of the pots with barren soil and pots planted with *Tar. odorata* (A), *Tristan. whiteana* (B), and *Tristel. australasiae* (C) control and N10 plants over 7 weeks. Weeks 1 – 3 were establishment weeks while weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

4.8 Water quality improvement

Pots that were planted with *Bhe. paniculata* or *Tal. tiliaceum* plants planted in them showed improved nitrate removal during weeks 5 – 7 compared to pots with barren soil (Figures 26C and 28F).

The amount of nitrate removed by pots planted with *Cle. sumatranus* and *Hop. ferrea* plants was only significantly higher than the amount of nitrate removed by the soil alone at the beginning of N10 solution irrigation (Figures 26G and 27D).The pots planted with *Bac. minor* and *Pla. obovata* plants only showed significantly higher nitrate removal compared to the soil alone in week 5 (Figures 26A and 27H). The amount of nitrate removed by pots planted with *Lit. sundaicus*, *Syz. myrtifolium*, and *Tristel. australasiae* plants

was significantly higher than the soil alone only in week 6 (Figures 27E, 28E and 29). The amount of nitrate removed by pots planted with *Ela. tapos* was significantly higher than the amount of nitrate removed by the soil alone by week 7 (Figure 27B). Although the nitrate removal by the soil alone was significantly higher initially, the nitrate removal by pots planted with *Gar. tubifera* and *Syz. leucoxylon* eventually became significantly higher than the soil alone by week 7 (Figures 27C and 28D).

The amount of nitrate removed by pots planted with *Bar. asiatica*, *Che. speciosus*, *Cri. asiaticum*, *Pae. foetida*, *Pip. sarmentosum*, *Sch. elliptica*, *Ste. macrophylla*, and *Tristan. whiteana* plants were not significantly different from the amount of nitrate removed by the soil alone across all treatment weeks (Figures 26B, F, and H, 27F and G, and 28B, C, and H).

Although the barren soil showed significantly higher nitrate removal initially, the amount of nitrate removed by the systems planted with *Bhe. robusta*, *Cal. longifolia*, *Pre. serratifolia*, and *Tar. odorata* increased to the same level as the soil by week 7 (Figures 26D and E, 28A and G).

The amount of nitrate removed by the soil was significantly higher than pots planted with *Dip. kerrii* for all treatment weeks (Figure 27A).

Lastly, the amount of phosphate removed by pots planted with any species was not significantly different than that of the soil alone throughout all weeks (Figures 26 – 29).

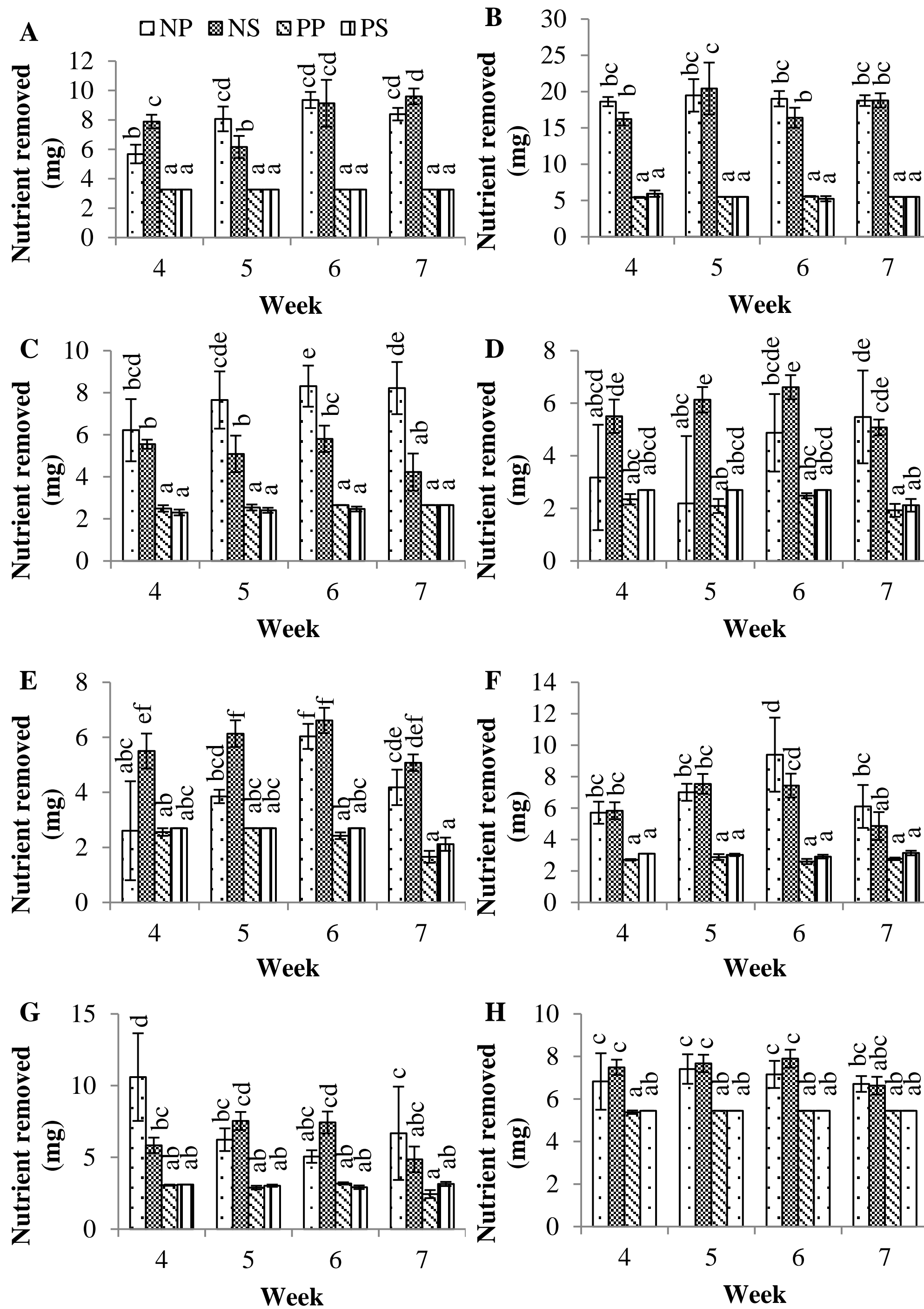

Figure 26. The nitrate (NP) and phosphate (PP) removal of pots planted with *Bac. minor* (A), *Bar. asiatica* (B), *Bhe. paniculata* (C), *Bhe. robusta* (D), *Cal. longifolia* (E), *Che. speciosus* (F), *Cle. sumatranus* (G), and *Cri. asiaticum* (H) plants over 7 weeks, as well as the nitrate (NS) and phosphate (PS) removal of the pots with barren soil. Weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

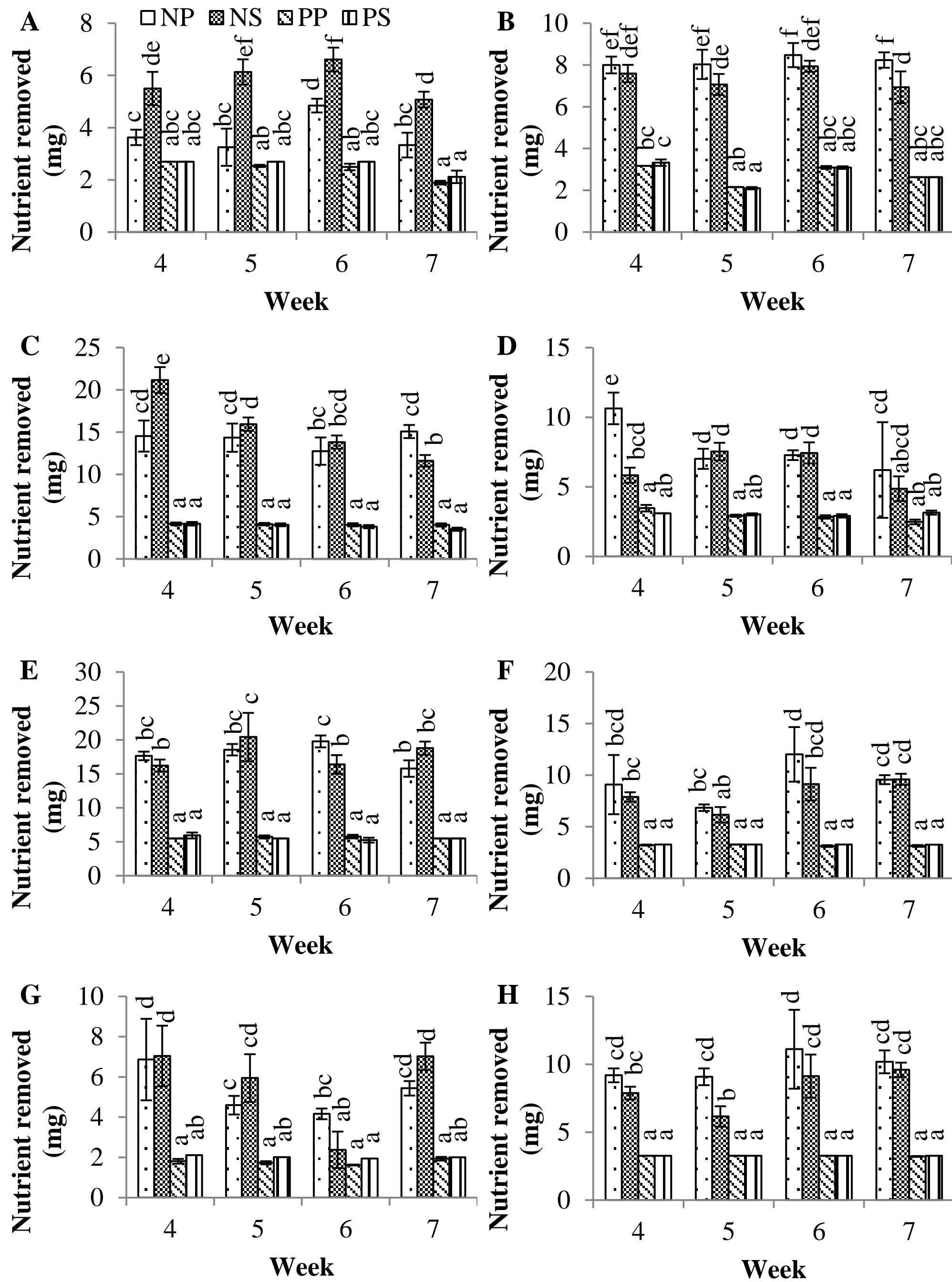

Figure 27. The nitrate (NP) and phosphate (PP) removal of pots planted with *Dip. kerrii* (A), *Ela. tapos* (B), *Gar. tubifera* (C), *Hop. ferrea* (D), *Lit. sundaicus* (E), *Pae. foetida* (F), *Pip. sarmentosum* (G), and *Pla. obovata* (H) plants over 7 weeks, as well as the nitrate (NS) and phosphate (PS) removal of the pots with barren soil. Weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

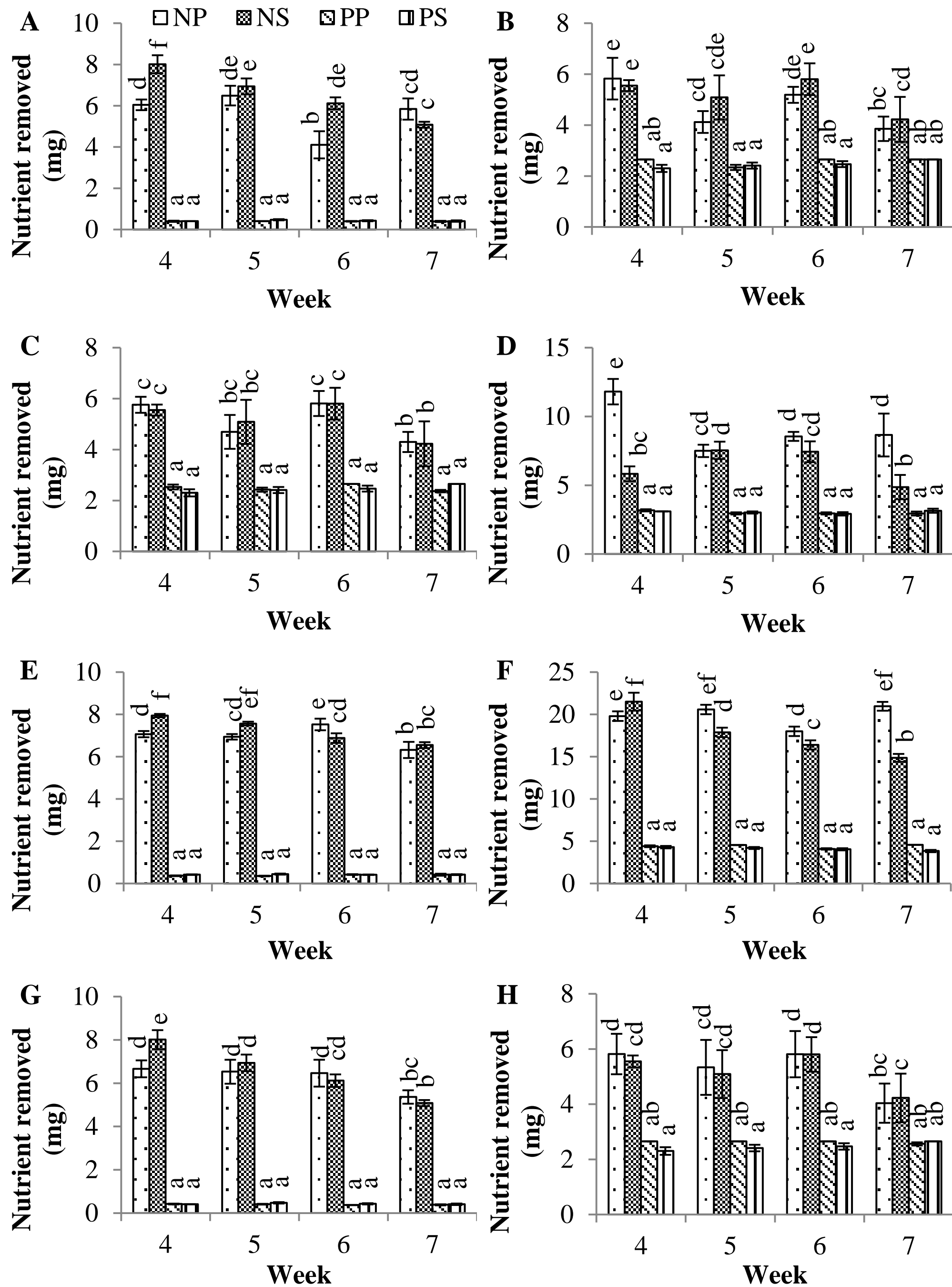

Figure 28. The nitrate (NP) and phosphate (PP) removal of pots planted with *Pre. serratifolia* (A), *Sch. elliptica* (B), *Ste. macrophylla* (C), *Syz. leucoxylon* (D), *Syz. myrtifolium* (E), *Tal. tiliaceum* (F), *Tar. odorata* (G), and *Tristan. whiteana* (H) plants over 7 weeks, as well as the nitrate (NS) and phosphate (PS) removal of the pots with barren soil. Weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates except for *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

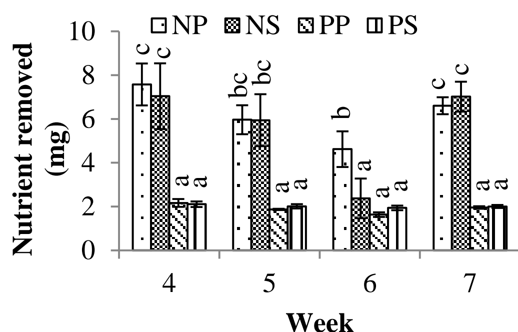

Figure 29. The nitrate (NP) and phosphate (PP) removal of pots planted with *Tristel. australasiae* plants over 7 weeks, as well as the nitrate (NS) and phosphate (PS) removal of the pots with barren soil. Weeks 4 – 7 were treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

Table 3. Summary of the range of percentage nitrate and phosphate removal recorded for each plant species, arranged in descending order according to highest nitrate removal. Plant species without a range of phosphate removal showed 100% for all weeks.

No.	Plant species	Nitrate removal (%)	Phosphate removal (%)
1	*Tal. tiliaceum*	51.02 – 59.42	89.49 – 100.00
2	*Syz. leucoxylon*	33.65 – 52.84	94.92 – 100.00
3	*Pae. foetida*	29.62 – 52.02	95.67 – 100.00
4	*Pla. obovata*	39.32 – 48.11	98.72 – 100.00
5	*Hop. ferrea*	27.78 – 47.60	80.12 – 100.00
6	*Cle. sumatranus*	22.62 – 47.41	78.58 – 100.00
7	*Syz. myrtifolium*	37.36 – 46.60	81.14 – 95.84
8	*Cri. asiaticum*	41.69 – 46.04	98.71 – 100.00
9	*Lit. sundaicus*	35.59 – 44.58	100.00
10	*Bar. asiatica*	41.95 – 43.88	98.80 – 100.00
11	*Tristel. australasiae*	26.20 – 42.96	71.34 – 94.50
12	*Gar. tubifera*	36.16 – 42.75	87.92 – 90.84
13	*Che. speciosus*	25.54 – 42.04	83.93 – 93.13
14	*Tar. odorata*	33.22 – 41.26	82.91 – 94.79
15	*Bac. minor*	24.57 – 40.50	100.00
16	*Pre. serratifolia*	25.42 – 40.20	89.26 – 92.54
17	*Ela. tapos*	32.00 – 39.65	100.00
18	*Pip. sarmentosum*	23.59 – 38.91	84.51 – 91.97
19	*Bhe. paniculata*	28.01 – 36.15	89.22 – 100.00
20	*Sch. elliptica*	23.81 – 35.97	88.22 – 100.00
21	*Tristan. whiteana*	24.93 – 35.91	96.55 – 100.00
22	*Ste. macrophylla*	26.57 – 35.86	89.39 – 100.00
23	*Bhe. robusta*	11.48 – 32.48	62.57 – 100.00
24	*Cal. longifolia*	13.70 – 31.70	61.54 – 100.00
25	*Dip. kerrii*	17.09 – 25.47	70.27 – 100.00

4.9 pH and conductivity of the leachate

The pH of the leachate from the pots of barren soil and planted pots, as well as pots irrigated with tap water or N10 solution, were all close to neutral throughout all treatment weeks, despite some significant differences (Figures 30, 31, 32, and 36). Although some differences could be observed between the groups, the differences were very small and the pH of the leachate were all still close to neutral.

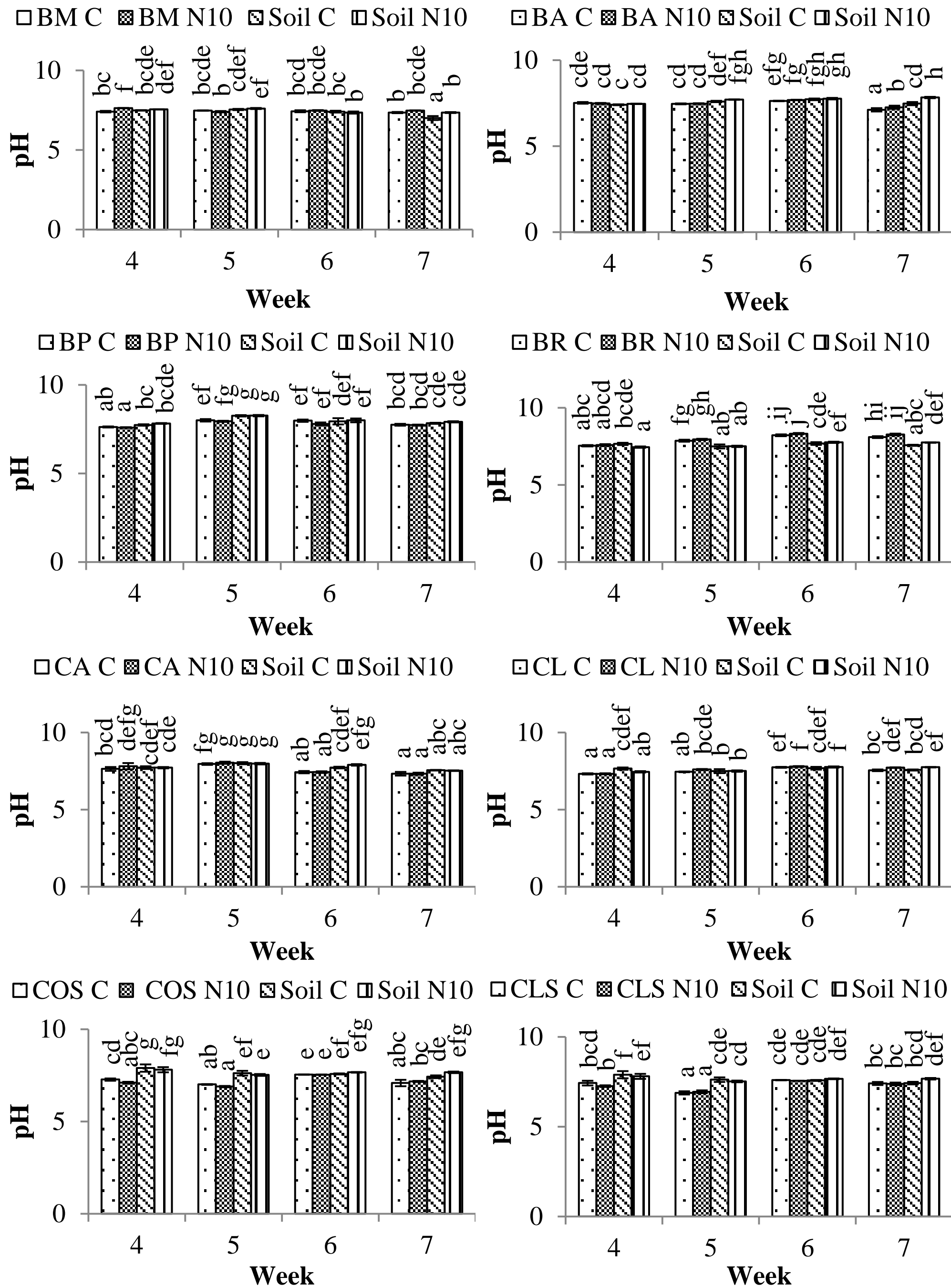

Figure 30. The pH of the leachate from pots planted with *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), and *Cle. sumatranus* (CLS) control (C) and N10 plants as well as from the pots with barren soil recorded during the treatment weeks. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

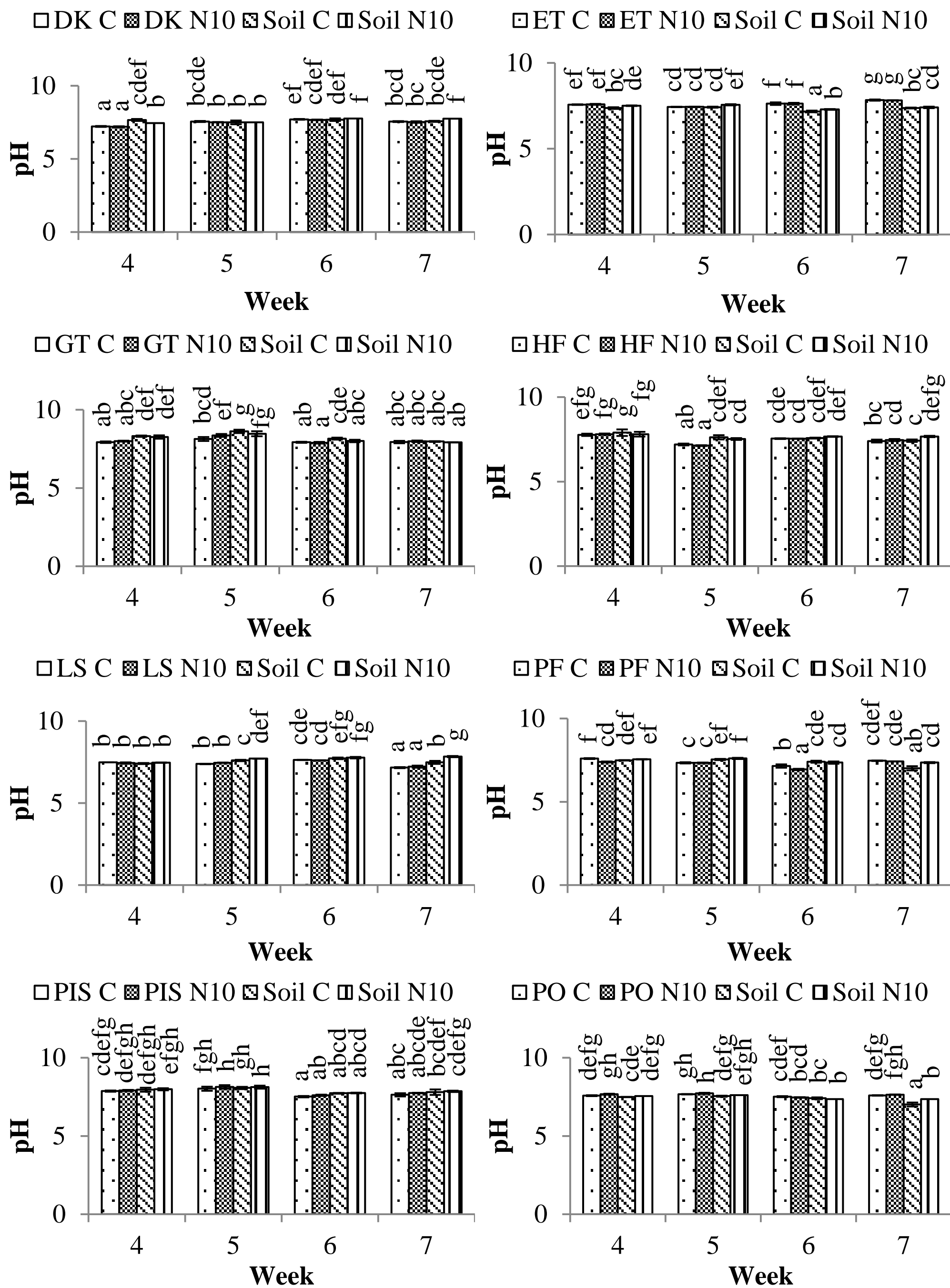

Figure 31. The pH of the leachate from pots planted with *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), and *Pla. obovata* (PO) control (C) and N10 plants as well as from the pots with barren soil recorded during the treatment weeks. Each bar represents the mean of 5 replicates except for *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

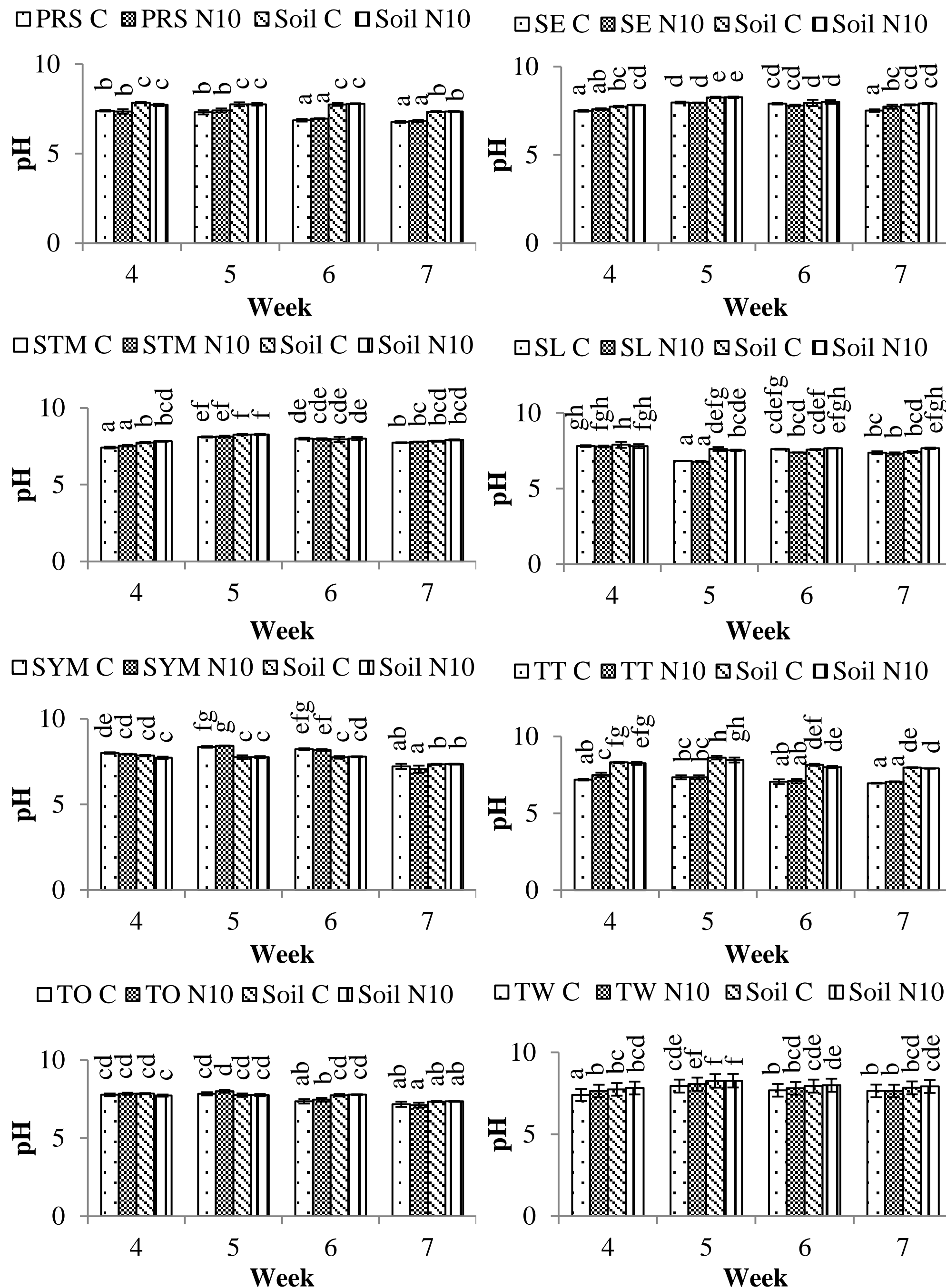

Figure 32. The pH of the leachate from pots planted with *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), and *Tristan. whiteana* (TW) control (C) and N10 plants as well as from the pots with barren soil recorded during the treatment weeks. Each bar represents the mean of 5 replicates except for *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

Overall, the conductivity of leachates from all pots showed a similar trend of an initially high conductivity which gradually decreased over the weeks (Figures 33, 34, 35, and 36). Most vegetated pots did not show a significant difference between pots watered with tap water or N10 solution, and pots with and without vegetation. This included pots planted with *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Cri. asiaticum*, *Cal. longifolia*, *Che. speciosus*, *Dip. kerrii*, *Ela. tapos*, *Hop. ferrea*, *Pae. foetida*, *Pla. obovata*, *Pre. serratifolia*, *Sch. elliptica*, *Ste. macrophylla*, *Syz. leucoxylon*, and *Tristan. whiteana* (Figures 33, 34, and 35).

Pots that were planted with *Bar. asiatica*, *Cle. sumatranus*, *Gar. tubifera*, *Lit. sundaicus*, *Pip. sarmentosum*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, and *Tristel. australasiae* had significantly lower conductivity by week 7 compared to pots with barren soil (Figures 33, 34, 35, and 36).

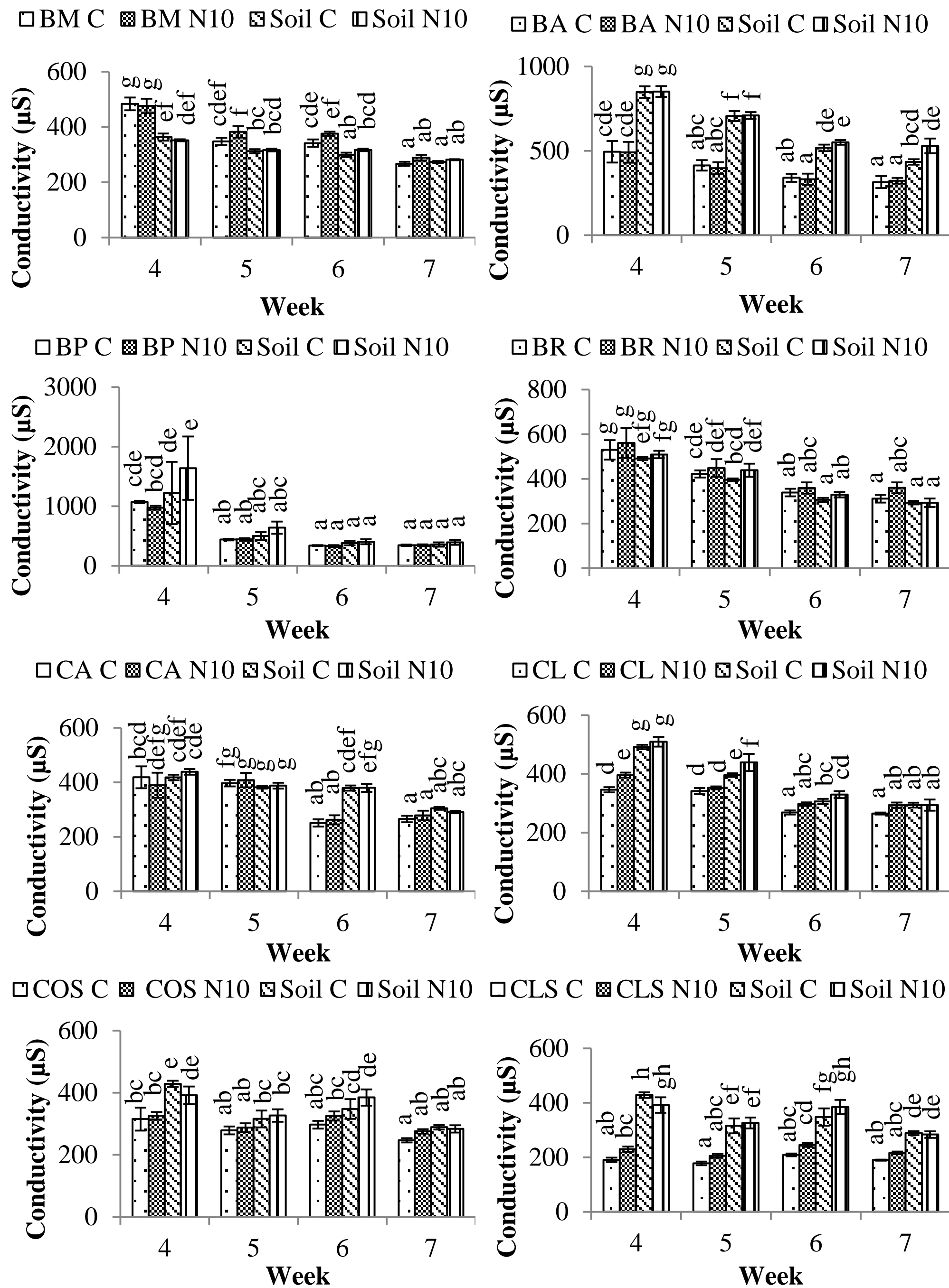

Figure 33. The conductivity of the leachates from pots planted with *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), and *Cle. sumatranus* (CLS) control (C) and N10 plants as well as from the pots with barren soil recorded during the treatment weeks. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

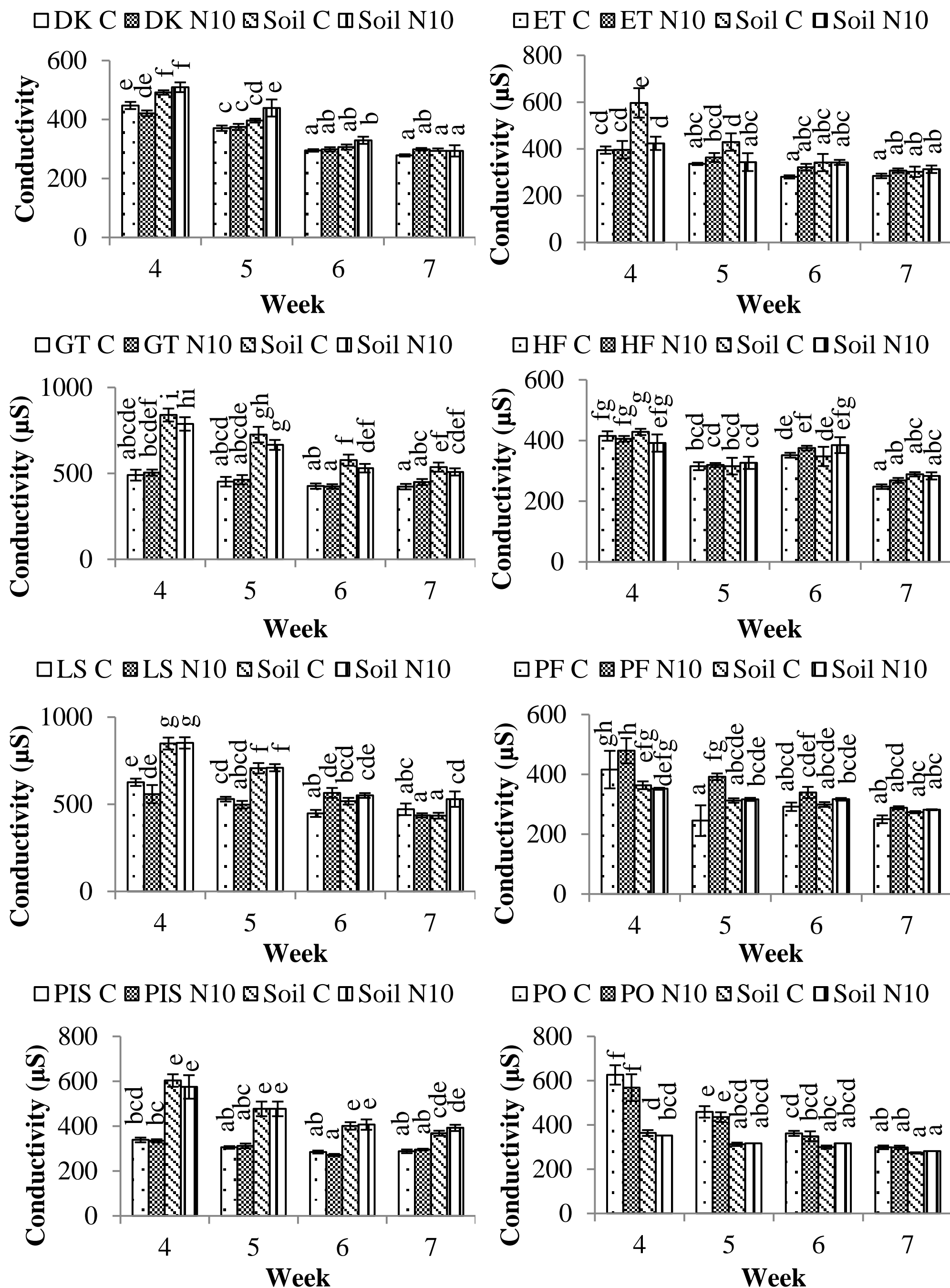

Figure 34. The conductivity of the leachates from pots planted with *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), and *Pla. obovata* (PO) control (C) and N10 plants as well as from the pots with barren soil recorded during the treatment weeks. Each bar represents the mean of 5 replicates except for *Hop. ferrea* plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

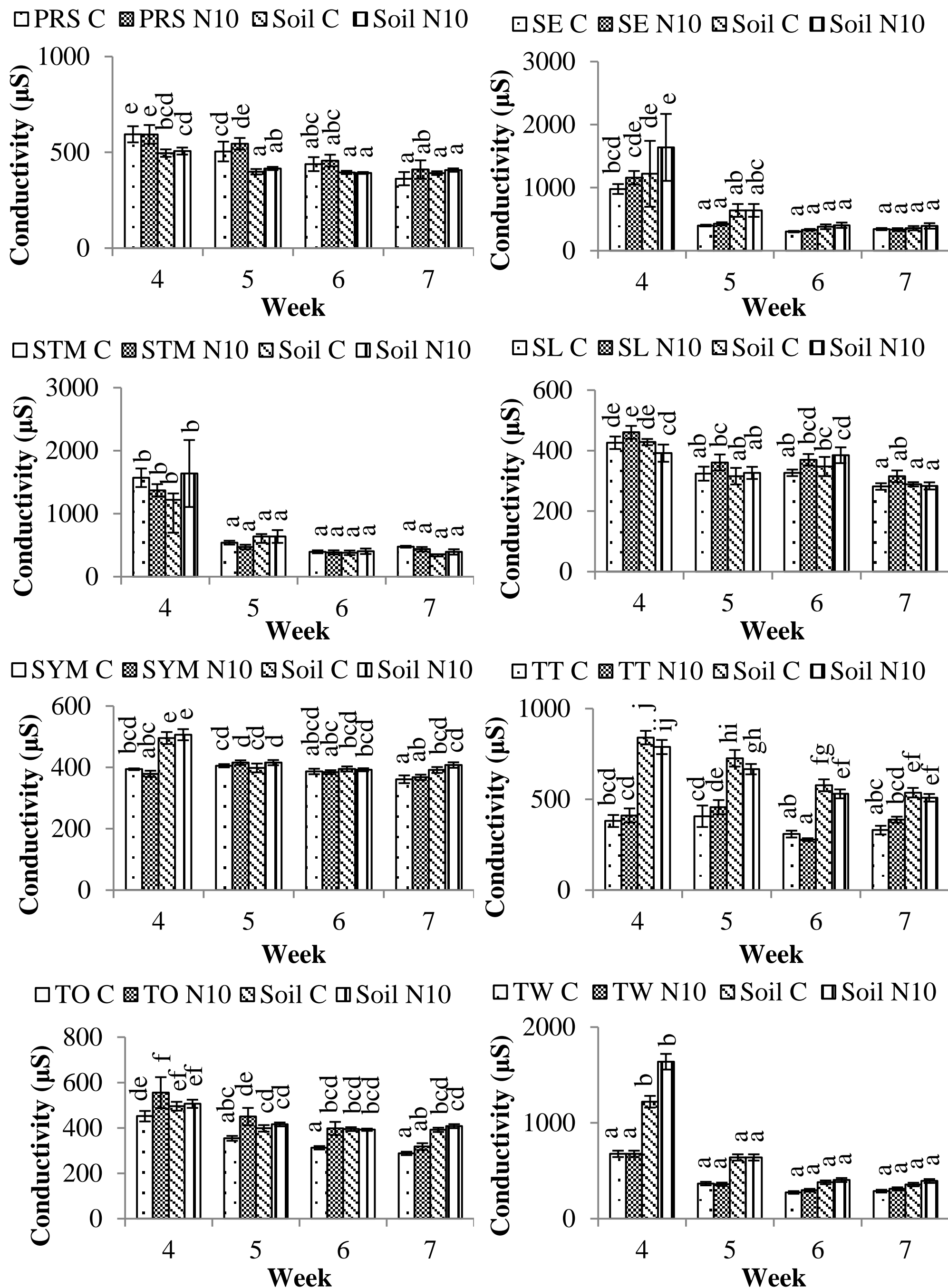

Figure 35. The conductivity of the leachates from pots planted with *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), and *Tristan. whiteana* (TW) control (C) and N10 plants as well as from the pots with barren soil recorded during the treatment weeks. Each bar represents the mean of 5 replicates except for *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

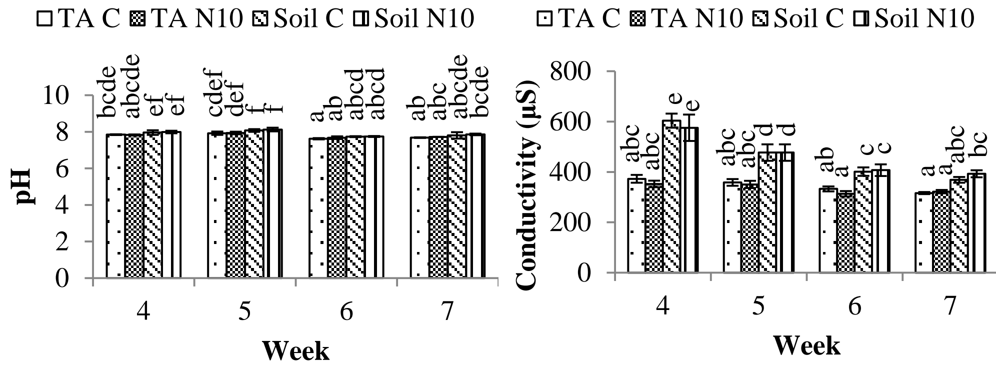

Figure 36. The pH and conductivity of the leachates from pots planted with *Tristel. australasiae* (TA) control (C) and N10 plants as well as from the pots with barren soil recorded during the treatment weeks. Each bar represents the mean of 5 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test.

4.10 Flow rate

The flow rate of water is presented as the percentage of total exfiltration over a period of 30 minutes. Overall, the planted pots and the pots with barren soil did not differ much from the start and end of treatment. The only pots which showed a marked difference in flow rate from week 4 to 7 were the big pots of soil which took a longer time for the total exfiltration to be complete at week 7 compared to week 4 (Figure 37). However, the total exfiltration was still completed within 60 minutes.

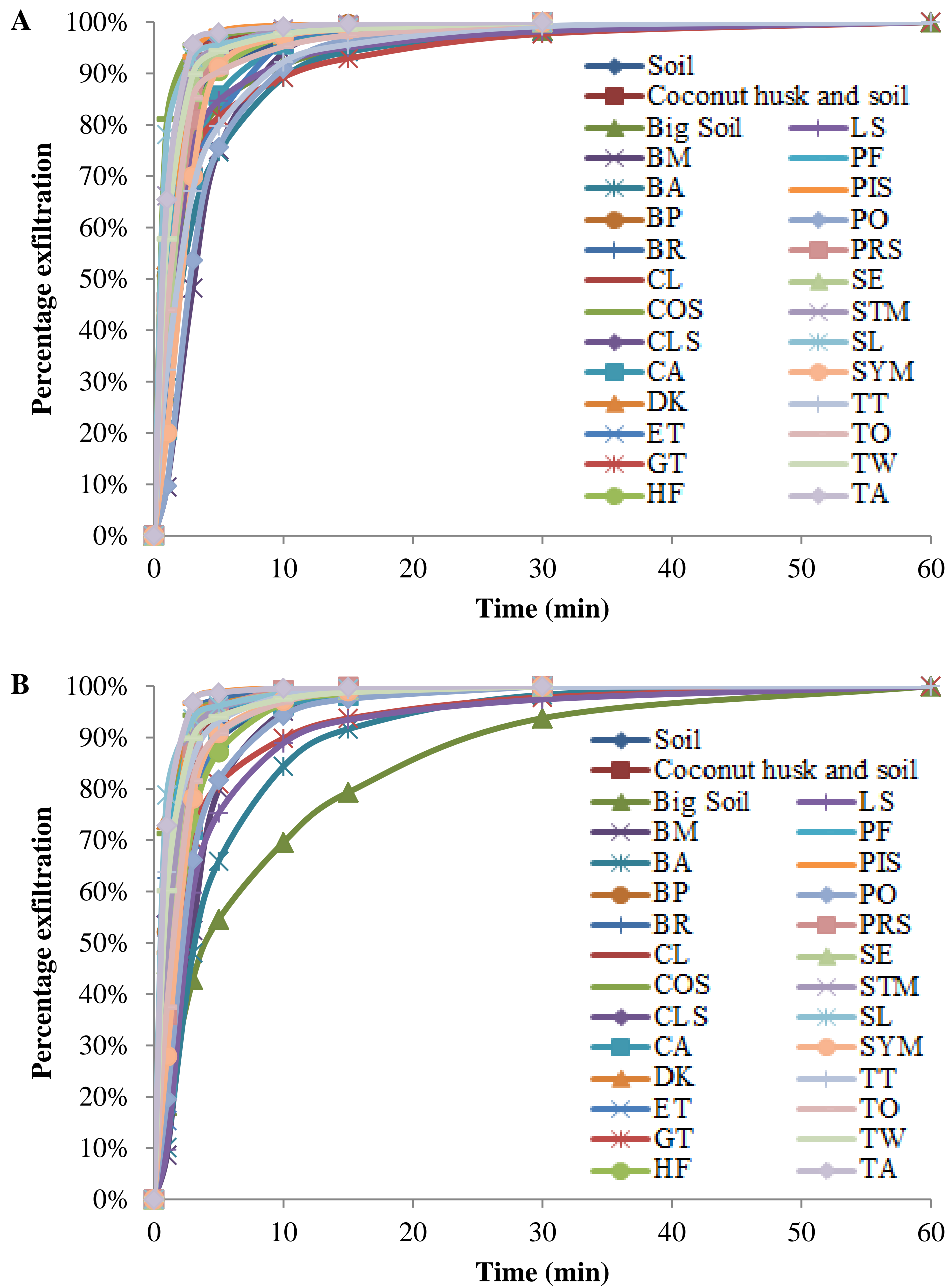

Figure 37. The exfiltration from pots of barren soil, coconut husk and barren soil, big pots of barren soil, and pots planted with *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) at the start of week 4 (A) and the end of week 7 (B).

4.11 Chlorophyll concentration correlated to SPAD

The ANOVA table (Table 4) shows that for all plant species, there was a statistically significant relationship between total chlorophyll concentration and SPAD values. *Bac. minor*, *Bar. asiatica*, *Bhe. paniculata*, *Bhe. robusta*, *Cal. longifolia*, *Che. speciosus*, *Cri. asiaticum*, *Ela. tapos*, *Gar. tubifera*, *Hop. ferrea*, *Lit. sundaicus*, *Pae. foetida*, *Pip. sarmentosum*, *Pla. obovata*, *Pre. serratifolia*, *Sch. elliptica*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, *Tristan. whiteana*, and *Tristel. australasiae* showed a statistically significant relationship at the 99% confidence level, whereas *Cle. sumatranus*, *Dip. kerrii*, and *Ste. macrophylla* were statistically significant at the 95% confidence level (Table 4).

Table 4. The regression analysis of fitting a linear model to describe the relationship between total chlorophyll concentration and SPAD.
** represents a statistically significant relationship at the 99% confidence level since the p-value is less than 0.01.
* represents a statistically significant relationship at the 95% confidence level since the p-value is less than 0.05.

Plant species	Analysis of Variance		
	F	*p*	
Bac. minor	72.54	0.0010	**
Bar. asiatica	150.03	0.0003	**
Bhe. paniculata	36.44	0.0009	**
Bhe. robusta	129.01	0.0015	**
Cal. longifolia	71.05	0.0035	**
Che. speciosus	50.26	0.0058	**
Cle. sumatranus	13.15	0.0361	*
Cri. asiaticum	25.08	0.0074	**
Dip. kerrii	9.52	0.0367	*
Ela. tapos	177.61	0.0009	**
Gar. tubifera	54.23	0.0007	**
Hop. ferrea	37.83	0.0035	**
Lit. sundaicus	31.49	0.0050	**
Pae. foetida	25.69	0.0071	**
Pip. sarmentosum	92.83	0.0006	**
Pla. obovata	129.39	0.0003	**
Pre. serratifolia	24.38	0.0026	**
Sch. elliptica	42.00	0.0013	**
Ste. macrophylla	23.62	0.0166	*
Syz. leucoxylon	91.24	0.0002	**
Syz. myrtifolium	54.20	0.0003	**
Tal. tiliaceum	198.17	0.0001	**
Tar. odorata	59.61	0.0002	**
Tristan. whiteana	74.49	0.0010	**
Tristel. australasiae	49.53	0.0021	**

4.12 Dry weight and specific leaf area (SLA)

The dry weight is presented as dry weight of different plant organs as well as the total plant dry mass. The dry mass of the reproductive parts of the plants was usually very small compared to the other organs and total dry mass, thus it was not presented individually but instead presented as part of the total dry weight. Although *Cri. asiaticum* is a bulbous plant and should only have leaves and roots, the leaves were sheathed together during development and thus are presented as "stems" in Figure 38.

The dry weights of *Bac. minor*, *Bar. asiatica*, *Bhe. paniculata*, *Bhe. robusta*, *Cal. longifolia*, *Che. speciosus*, *Cle. sumatranus*, *Cri. asiaticum*, *Dip. kerrii*, *Gar. tubifera*, *Hop. ferrea*, *Pae. foetida*, *Pip. sarmentosum*, *Pla. obovata*, *Sch. elliptica*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, and *Tristan. whiteana* control and N10 plants did not differ significantly by specific organ or total dry mass (Figure 38). *Ela. tapos* control plants had significantly higher total dry mass than N10 plants (Figure 38). *Pre. serratifolia* control plants had significantly higher root mass and subsequently total mass compared to N10 plants (Figure 38). Lastly, *Lit. sundaicus* and *T. australasia* N10 plants showed significantly higher total dry mass compared to control plants (Figure 38).

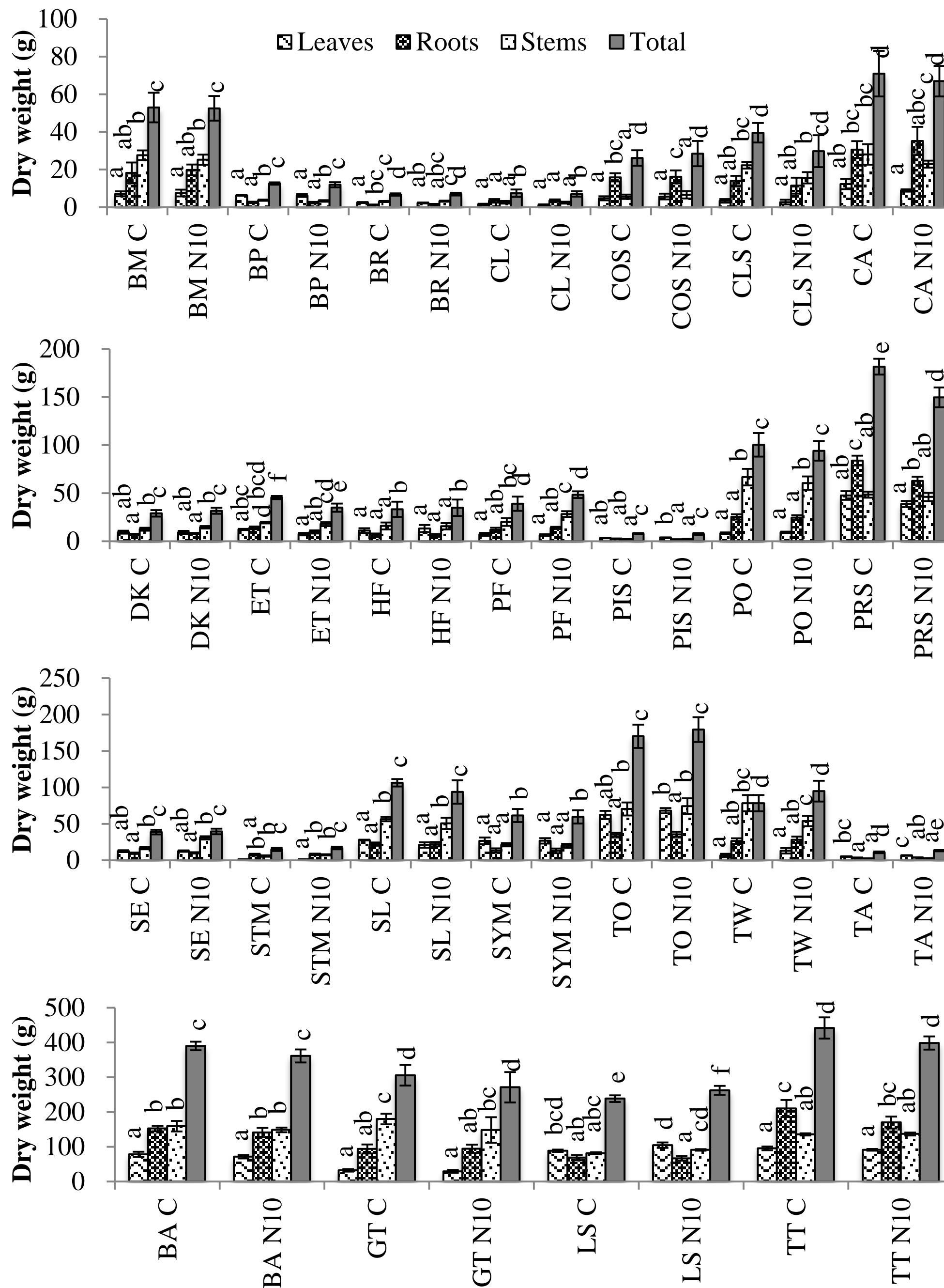

Figure 38. The dry weights of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants after harvest. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 within the same species.

The SLA of the control and N10 plants were not significantly different for *Bar. asiatica*, *Bhe. paniculata*, *Bhe. robusta*, *Cal. longifolia*, *Che. speciosus*, *Cle. sumatranus*, *Dip. kerrii*, *Ela. tapos*, *Gar. tubifera*, *Hop. ferrea*, *Lit. sundaicus*, *Pae. foetida*, *Pip. sarmentosum*, *Pla. obovata*, *Pre. serratifolia*, *Sch. elliptica*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, *Tristan. whiteana*, and *T. australasia* (Figure 39). The only two species which showed a difference in SLA when irrigated with different solutions are *Bac. minor* and *Cri. asiaticum*. *Bac. minor* control plants were significantly lower than N10 plants, whereas *Cri. asiaticum* N10 plants were significantly lower than control plants (Figure 39).

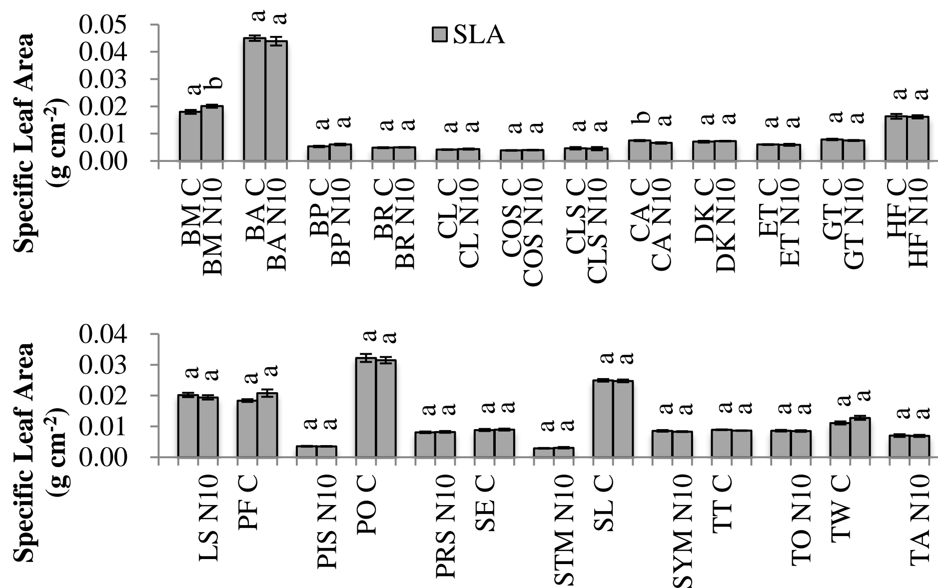

Figure 39. The SLA of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants after harvest. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 plants within the same species.

4.13 Total soluble protein concentration (TSP)

The concentration of total soluble proteins of *Bar. asiatica*, *Bhe. paniculata*, *Bhe. robusta*, *Cal. longifolia*, *Che. speciosus*, *Cle. sumatranus*, *Cri. asiaticum*, *Dip. kerrii*, *Ela. tapos*, *Gar. tubifera*, *Hop. ferrea*, *Lit. sundaicus*, *Pip. sarmentosum*, *Pla. obovata*, *Pre. serratifolia*, *Sch. elliptica*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, *Tristan. whiteana*, and *T. australasia* control and N10 plants were not significantly different (Figure 40). The only two species which showed a significant difference in TSP when irrigated with different solutions were *Bac. minor* and *Pae. foetida*, where plants irrigated with N10 solution showed lower TSP concentration compared to control plants (Figure 40).

The TSP for *Gar. tubifera* was not determined because the leaf samples produced a thick gelatinous layer during the protein extraction, resulting in a mixture that could not be separated even when centrifuged.

4.14 Total Kjeldahl Nitrogen (TKN)

The TKN determined was not significantly different for *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Cle. sumatranus*, *Cri. asiaticum*, *Dip. kerrii*, *Gar. tubifera*, *Hop. ferrea*, *Lit. sundaicus*, *Pae. foetida*, *Pip. sarmentosum*, *Pla. obovata*, *Pre. serratifolia*, *Sch. elliptica*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tristan. whiteana*, and *T. australasia* control and N10 plants (Figure 41).

The leaves of *Bar. asiatica* control plants showed higher TKN compared to the leaves of N10 plants (Figure 41). *Cal. longifolia* and *Ela. tapos* N10 plant leaves showed significantly higher TKN compared to the

leaves of control plants (Figure 41). And finally, the reproductive organs of *Tar. odorata* N10 plants showed significantly higher TKN compared to control plants (Figure 41).

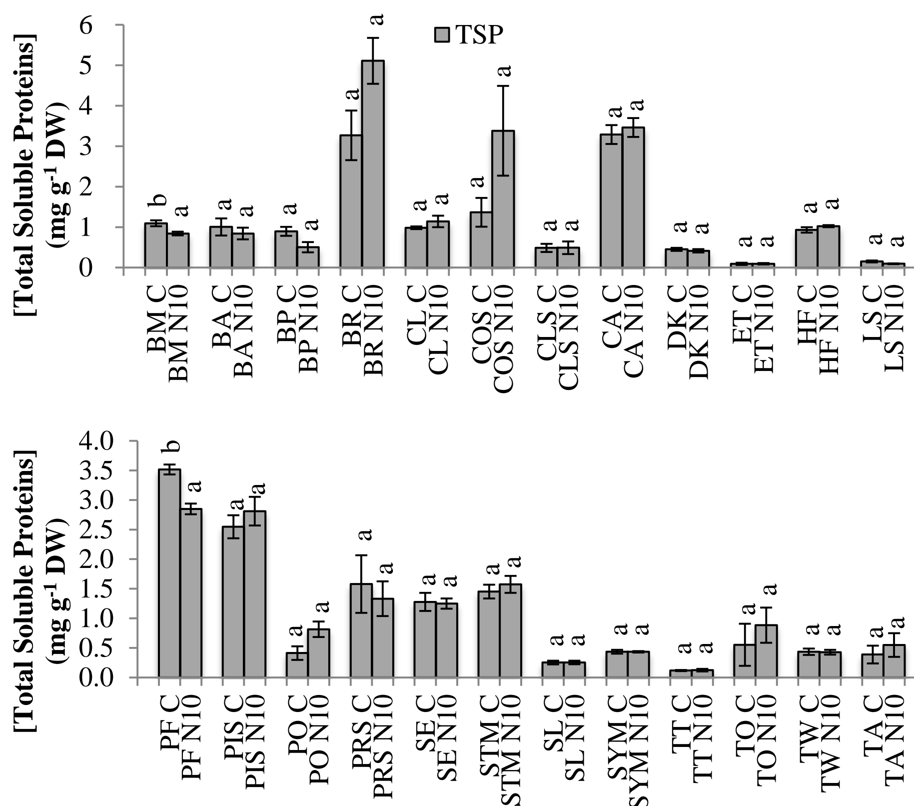

Figure 40. The TSP of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants after harvest. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 plants within the same species.

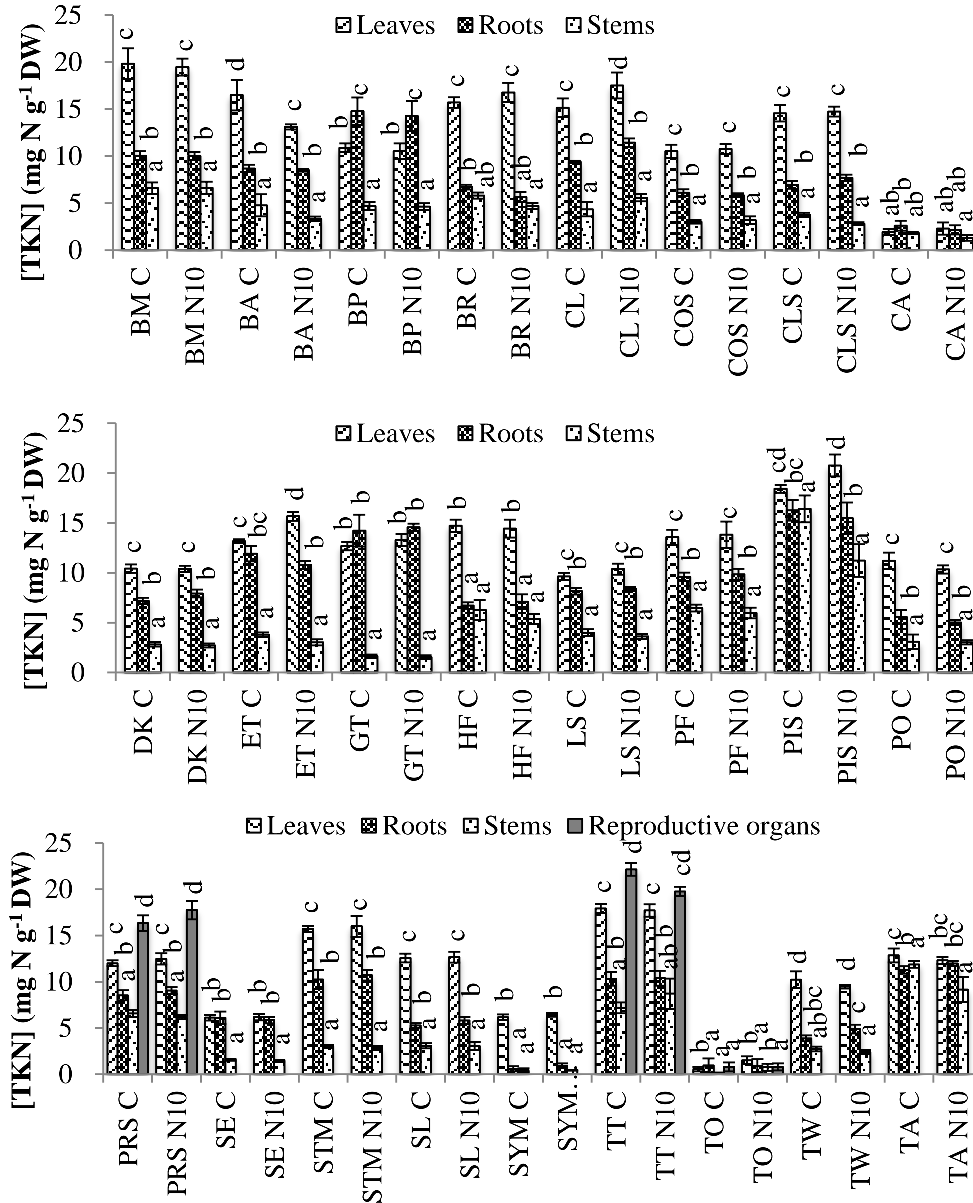

Figure 41. The TKN of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants after harvest. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 plants within the same species.

4.15 Total phosphorus concentration (TP)

The TP determined in control and N10 plants of *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Che. speciosus*, *Cle. sumatranus*, *Cri. asiaticum*, *Hop. ferrea*, *Pae. foetida*, *Pip. sarmentosum*, *Pla. obovata*, *Sch. elliptica*, *Ste. macrophylla*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, *Tristan. whiteana*, and *T. australasia* showed no significant differences (Figure 42).

Cal. longifolia and *Pre. serratifolia* N10 plants showed higher TP in the roots compared to roots of control plants (Figure 42). Conversely, *Bar. asiatica* control plants showed higher TP in the roots than N10 plants (Figure 42).

Dip. kerrii and *Ela. tapos* control plants had higher TP in the stems compared to N10 plants (Figure 42).

Gar. tubifera, *Lit. sundaicus*, and *Syz. leucoxylon* N10 plants showed higher TP in the leaves compared to the leaves of control plants (Figure 42).

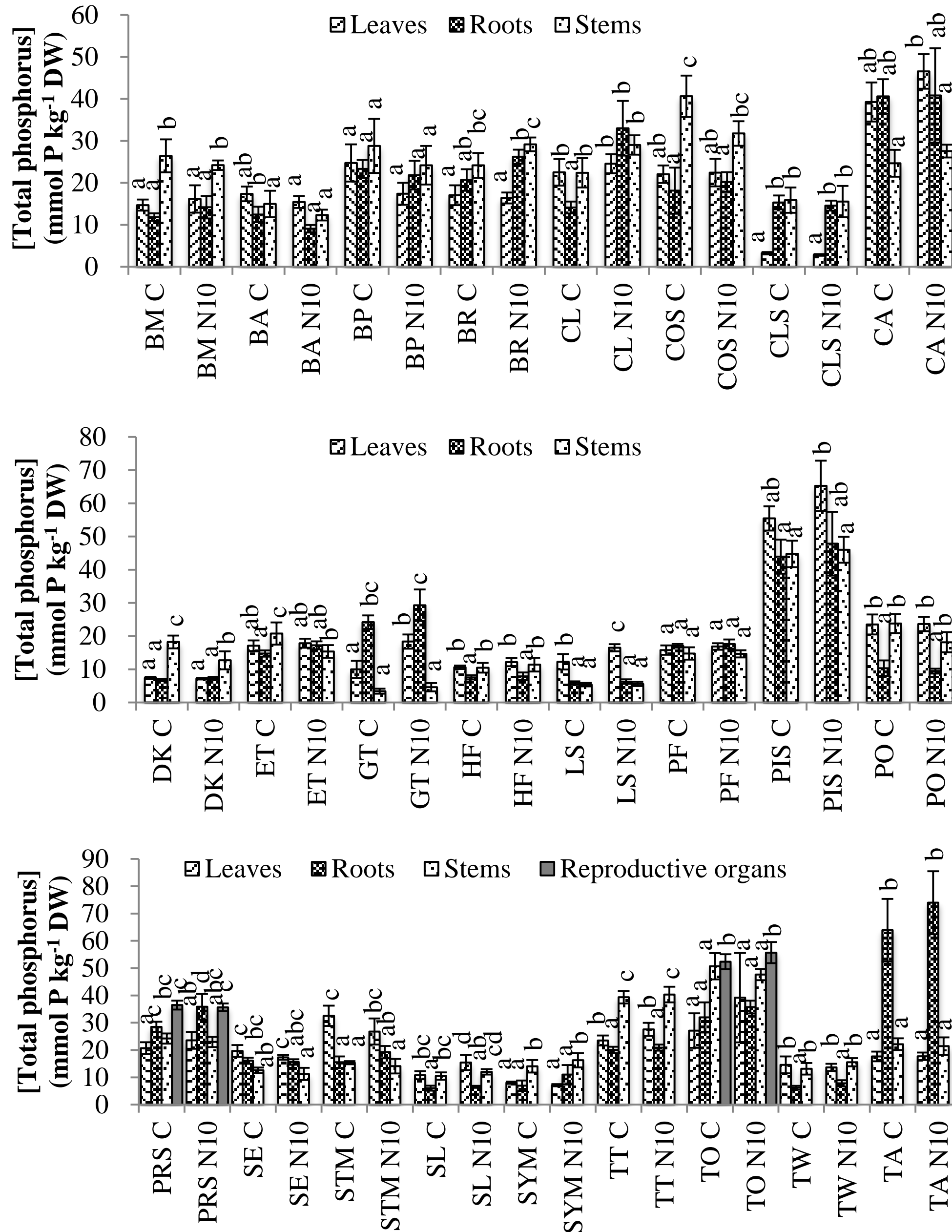

Figure 42. The TP of *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Cri. asiaticum* (CA), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) control (C) and N10 plants after harvest. Each bar represents the mean of 5 replicates except for *Bhe. paniculata* control plants, *Hop. ferrea* plants, and *Syz. leucoxylon* N10 plants which was a mean of 4 replicates. The error bars represent the standard error. The letters above each bar represent the statistical group after conducting a Fisher's least significant difference (LSD) test on C and N10 plants within the same species.

4.16 Root characteristics

From the dry weight results in Section 4.12, it was observed that control and N10 plants did not differ significantly in root mass except *Pre. serratifolia* (Figure 38). Even though there was a significant difference in the dry mass of *Pre. serratifolia* control and N10 plants, the root systems displayed the same characteristics. The same was also observed for the root systems within each species. Thus in this section, only one representative picture of the roots is presented. Root depth is not accurately displayed as the plants would have been limited to a fixed root depth due to the pot size.

Most of the plants showed thin, dense root systems, despite the different plant habit. *Bac. minor*, *Bhe. paniculata*, *Bhe. robusta*, *Cal. longifolia*, *Cle. sumatranus*, *Dip. kerrii*, *Ela. tapos*, *Gar. tubifera*, *Hop. ferrea*, *Lit. sundaicus*, *Pla. obovata*, *Ste. macrophylla*, *Syz. leucoxylon*, *Syz. myrtifolium*, and *Tristan. whiteana* were all tree species that showed thin, dense root systems (Plates 27A, C, D, E, F, I, J, K, L, and M and 28C, F, G, H, and K) similar to *Che. speciosus*, *Pae. foetida*, *Pip. sarmentosum*, *P. serratofolia*, *Sch. elliptica*, *Tar. odorata*, and *Tristel. australasiae* (Plates 27G and 28A, B, D, E, J, and L) which are shrubs, herbs, vines, or lianas. The plants that showed the thickest roots were *Bar. asiatica*, *Tal. tiliaceum*, and *Cri. asiaticum* (Plates 27B and H and 28I), two trees and a bulbous species respectively.

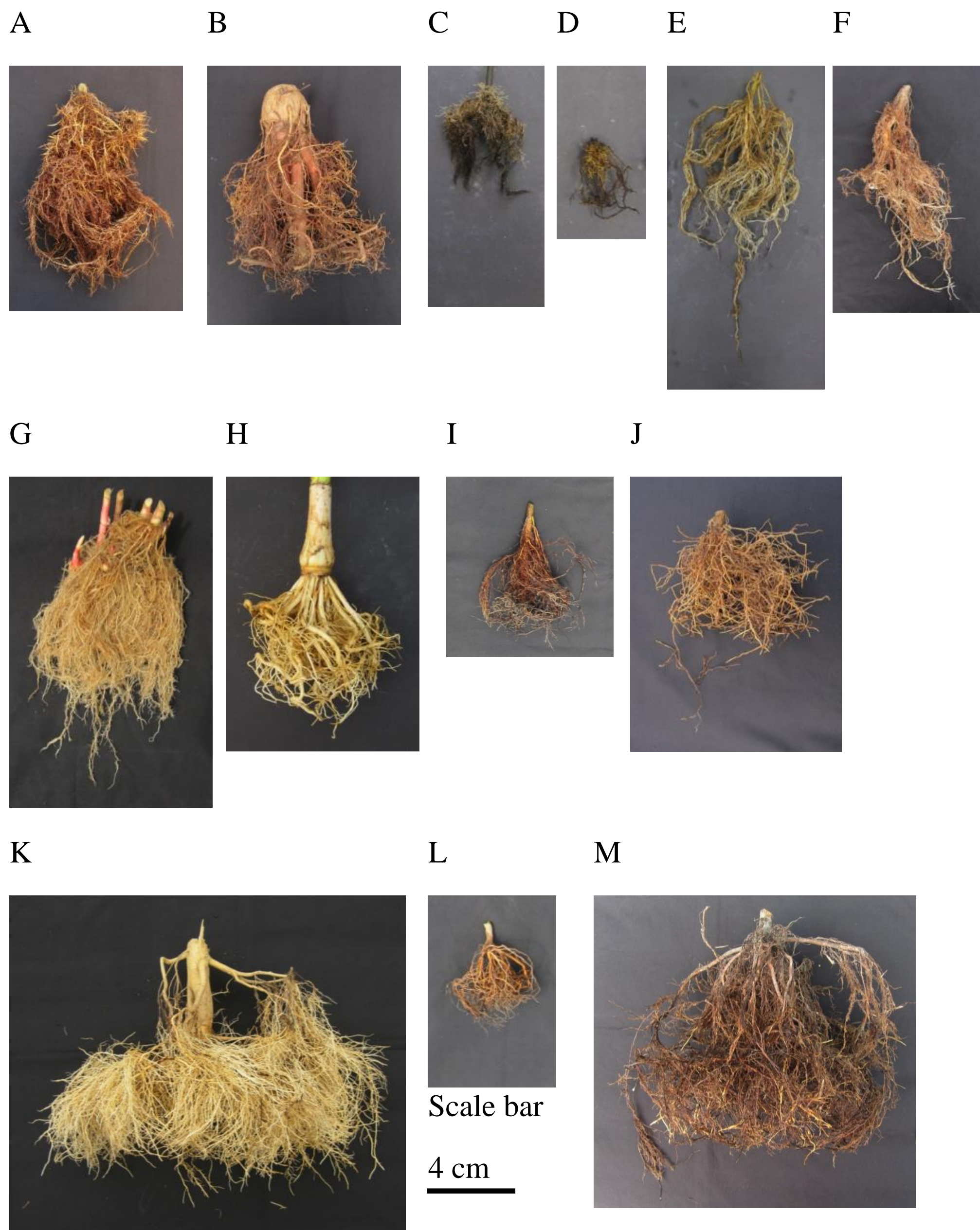

Plate 27. The roots of *Bac. minor* (A), *Bar. asiatica* (B), *Bhe. paniculata* (C), *Bhe. robusta* (D), *Cal. longifolia* (E), *Cle. sumatranus* (F), *Che. speciosus* (G), *Cri. asiaticum* (H), *Dip. kerrii* (I), *Ela. tapos* (J), *Gar. tubifera* (K), *Hop. ferrea* (L), and *Lit. sundaicus* (M) after harvest.

Plate 28. The roots of *Pae. foetida* (A), *Pip. sarmentosum* (B), *Pla. obovata* (C), *Pre. serratifolia* (D), *Sch. elliptica* (E), *Ste. macrophylla* (F), *Syz. leucoxylon* (G), *Syz. myrtifolium* (H), *Tal. tiliaceum* (I), *Tar. odorata* (J), *Tristan. whiteana* (K), and *Tristel. australasiae* (L) after harvest.

4.17 Nutrient removal related to plant traits

After studying the different nutrient removal efficiencies and plant traits, various analyses were conducted to understand how the different plant traits might influence nutrient removal. The mass (mg) of nitrate and phosphate removed were compared to the natural habitats the plants were found in. This showed that the amount of nutrient removed was not related to where the plants are usually naturally found (Figure 43). From Figure 43, the mass of nitrate and phosphate removal appeared to be more species-dependent instead of dependent on the species' natural habitat. The only natural habitat that seemed to affect nitrate and phosphate removal consistently in the different species was the forest edge, where *Che. speciosus*, *Ela. tapos*, and *Pae. foetida* all showed similar nutrient pollutant removal.

Some plant traits were also correlated to different important functions of the plants in a bioretention system such as nitrate removal, phosphate removal and flow rate. Root thickness was estimated from the photographs, and initial flow rate refers to the flow rate during the first 5 minutes of the flow rate experiments.

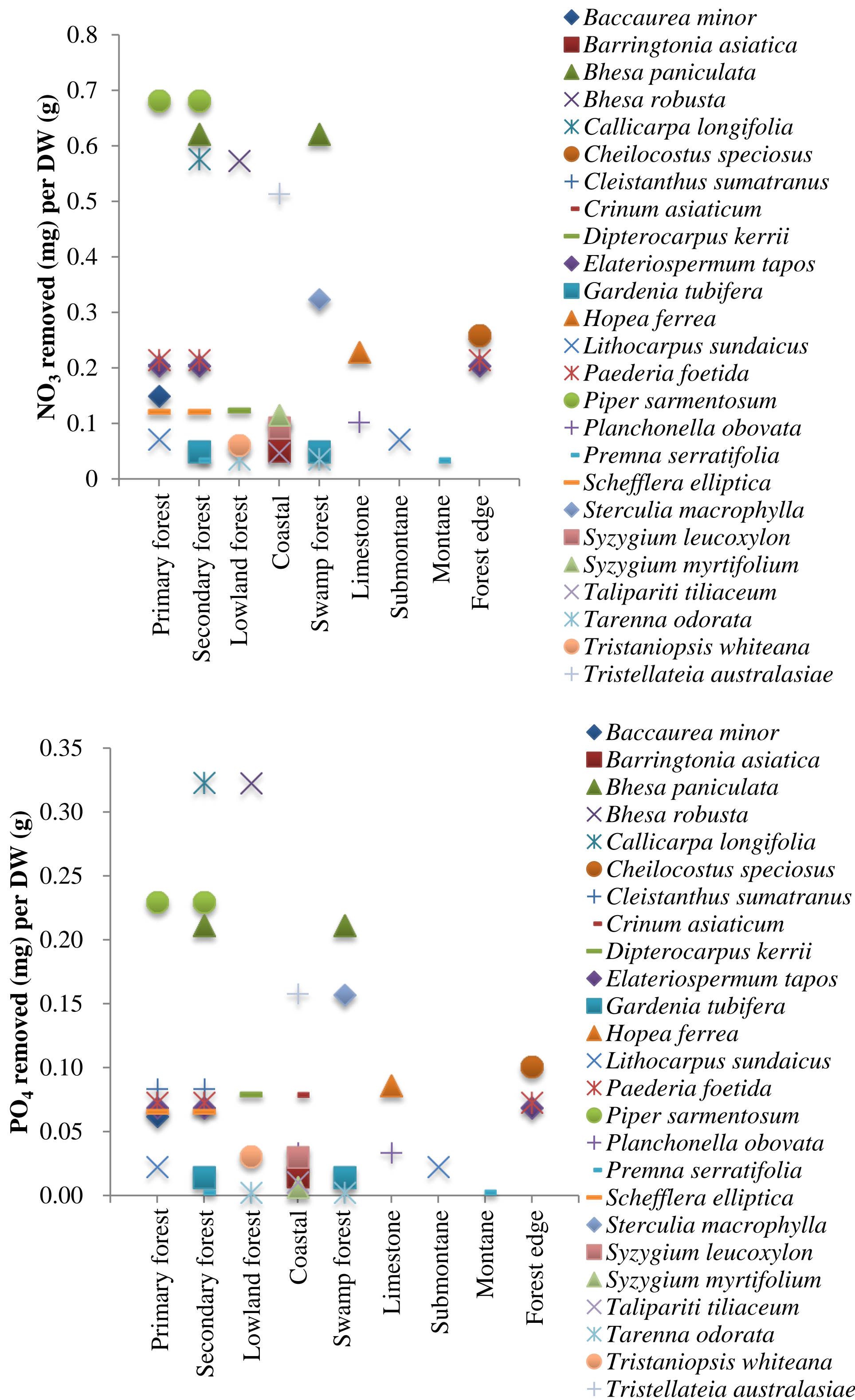

Figure 43. The amount of NO_3 and PO_4 removed per dry biomass of various species related to the species' natural habitat.

Overall, root dry mass and total plant dry mass were not related to nitrate and phosphate removal in non-tree species, whereas it was shown to be significantly related in tree species. Flow rate in tree species was not related to root dry mass, total plant dry mass, or root thickness, but in non-tree species it was significantly related to total plant dry mass and root thickness.

Strong correlations were found between dry weight and nitrate and phosphate removal in trees species. In tree species, root dry mass showed a statistically significant relationship with nitrate and phosphate removal at the 99% confidence level, explaining 81% and 51% of the variation in nitrate and phosphate removed respectively (Figure 44). However, the same was not observed for non-tree species. This linear relationship was even stronger when total plant dry mass was correlated to nitrate and phosphate removal in trees where 89% and 60% of the variation in nitrate and phosphate removal respectively could be explained by the total dry mass of the trees (Figure 45). Although the root dry mass was not significantly related to the flow rate in non-tree species, the total dry mass showed a significant relationship to flow rate at the 95% confidence level, and explained 60% of the variation in flow rate (Figure 45).

Lastly, root thickness in tree species showed a statistically significant relationship to nitrate and phosphate removal in trees at the 95% confidence level. Root thickness accounted for 32% and 33% of the variation observed in nitrate and phosphate removed respectively (Figure 46). Root thickness was also significantly related to flow rate in non-tree species at the 90% confidence level where it could account for 43% of the variation.

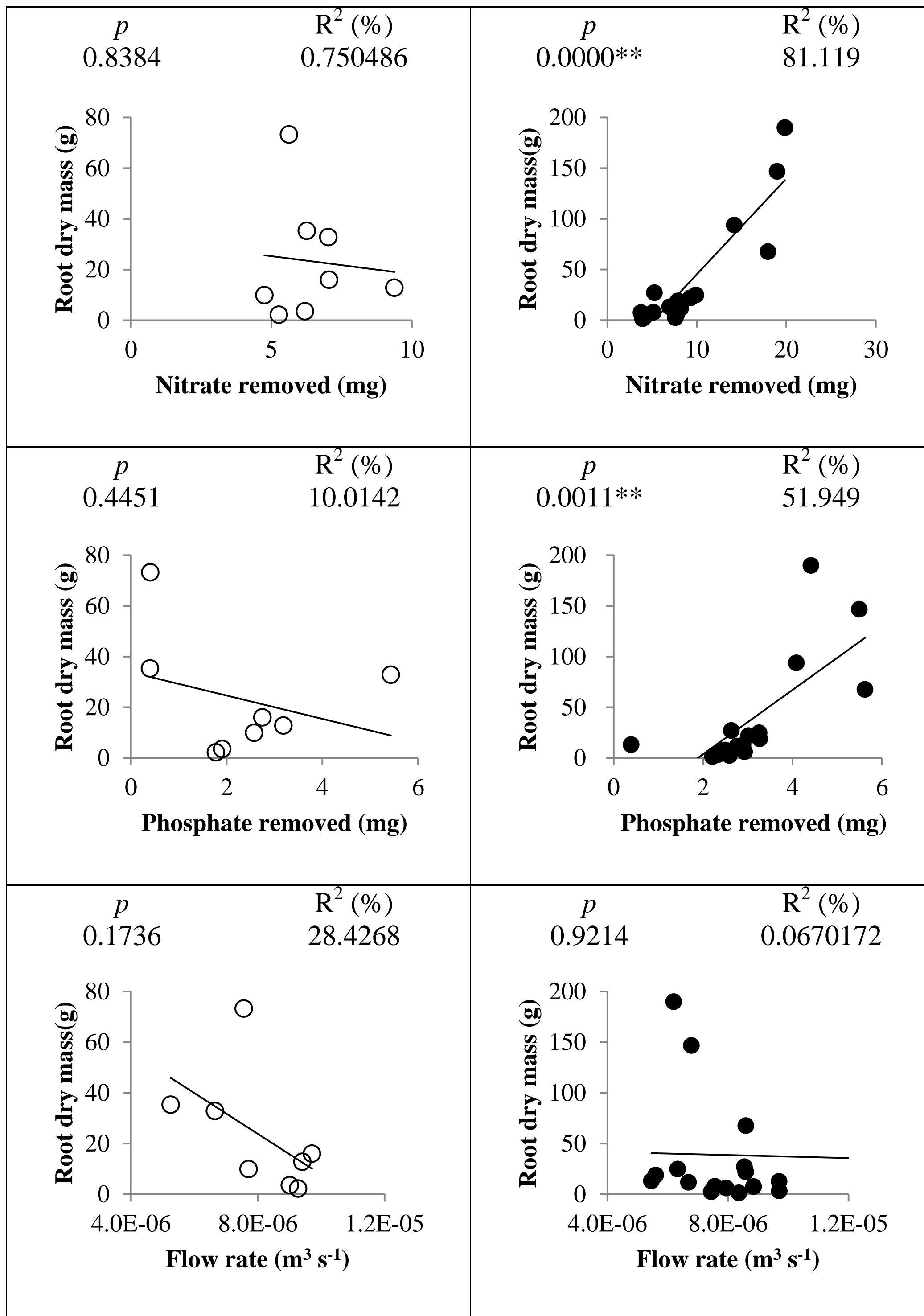

Figure 44. The correlations of root dry mass with nitrate removal, phosphate removal, and initial flow rate. Open symbols refer to non-tree species and filled symbols refer to tree species. The results of the linear regression are shown above the scatter plot.

** indicates significance at the 99% confidence level.
* indicates significance at the 95% confidence level.

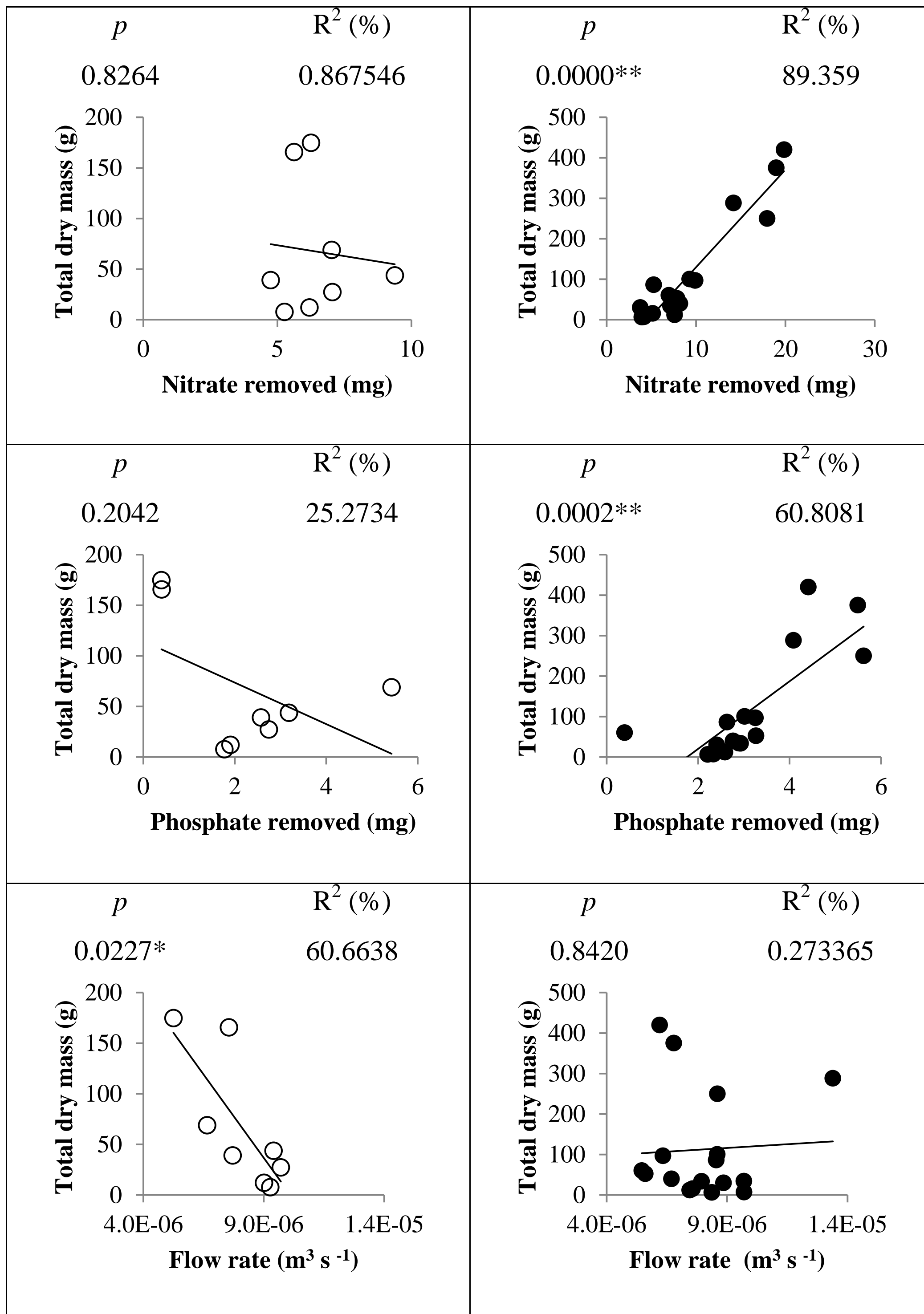

Figure 45. The correlations of total dry mass with nitrate removal, phosphate removal, and initial flow rate. Open symbols refer to non-tree species and filled symbols refer to tree species. The results of the linear regression are shown above the scatter plot.

** indicates significance at the 99% confidence level.
* indicates significance at the 95% confidence level.

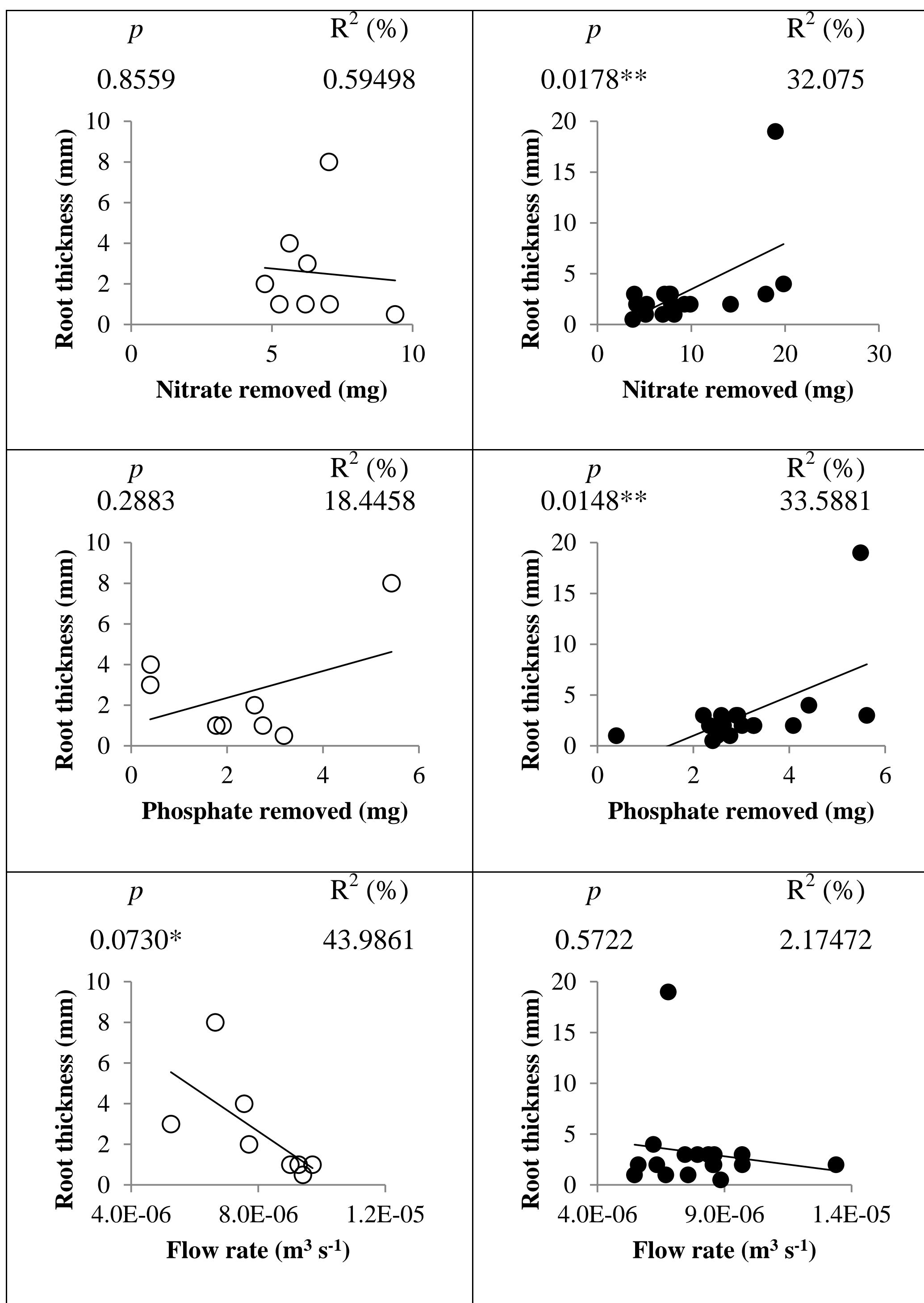

Figure 46. The correlations of root thickness with nitrate removal, phosphate removal, and initial flow rate. Open symbols refer to non-tree species and filled symbols refer to tree species. The results of the linear regression are shown above the scatter plot.

** indicates significance at the 95% confidence level.
* indicates significance at the 90% confidence level.

Chapter 5. Discussion

The suitability of plants for bioretention systems depend not only on the ability of the plants to remove pollutants and to maintain the growth/filter medium, but also on the ability of the plants to tolerate the abiotic conditions in the bioretention system. Bioretention systems have harsh abiotic conditions as they are usually situated in open areas to receive stormwater runoff, exposed to high light levels, and unpredictable precipitation. As bioretention systems are meant to be low maintenance, the plants have to withstand periods without watering if there is no natural precipitation, as well as the other extreme of periods of ponding and high soil moisture when the influx of stormwater runoff enters the system. Plants used in such bioretention systems must adapt to these harsh environmental conditions, and the present study was conducted to test the suitability of 25 native species to Singapore for planting in bioretention systems and phytoremediation of nitrate and phosphate. Some non-destructive parameters that could be used to determine plant health to test if the plants could tolerate the harsh conditions in the bioretention system were chlorophyll fluorescence, leaf greenness (SPAD), leaf growth, and visual assessment. The plants were allowed to establish in the new growth environment for 3 weeks before starting the experiments on phytoremediation. Plant health and growth were monitored throughout the experiments. Although one or two of each *Bhe. paniculata*, *Hop. ferrea*, and *Syz. leucoxylon* plants died after repotting, the majority of the plants (99%) were able to withstand the stress of repotting.

In addition to plant health and nutrient phytoremediation, there is the need to understand nutrient accumulation after plant uptake. Previous studies

on heavy metals had shown that pollutant translocation from the below- to above-ground plant parts was beneficial to permanently eliminate toxic elements (Bragato et al., 2009; Salem et al., 2014). In vanadium phytoremediation by *Artemisia vulgaris*, *Polygonum cuspidatum*, *Phragmites australis*, *Rhus copallinum*, *Betula populifolia*, and *Populus deltoides* plants in the United States of America, vanadium concentration was highest in the roots; the higher the concentration in the roots could be linked to the higher the soil potential to leach vanadium content (Qian et al., 2014). Nutrient pollutant accumulation in different plant parts from phytoremediation of stormwater runoff have not been reported before, although there are ample literature highlighting the importance of plants in bioretention systems to enhance nutrient pollutant removal (Hatt et al., 2007a; Henderson et al., 2007; Lucas and Greenway, 2008; Read et al., 2008; Read et al., 2010). In heavy metals, pollutant translocation to aboveground plant organs was important to prevent leaching back into the media (Bragato et al., 2009; Nunes da Silva et al., 2014; Salem et al., 2014), and thus the present study also aimed to understand the different nutrient allocation in the plants during nutrient pollutant removal from stormwater runoff. It is important that the nutrient pollutants taken up by the plants would not eventually leach back into the system, following the same principle for heavy metal phytoremediation. The pollutant accumulation in various plant parts were determined destructively after harvest in terms of dry weight, specific leaf area (SLA), total soluble proteins (TSP), total Kjeldahl nitrogen (TKN), and total phosphorus (TP).

5.1 Plant health and growth

Chlorophyll fluorescence has been used to determine plant response and stress in biofiltration systems experiments (Read et al., 2008). F_v/F_m readings are a sensitive indicator of physiological stress in plants and almost all the plants (92%) studied showed healthy F_v/F_m readings, ranging from 0.76 – 0.90 (Figures 2 – 5), indicating that the plants were not physiologically stressed by the irrigation with N10 solution. This is similar to the study by Read et al. (2008), who showed that the mean F_v/F_m readings for a variety of 20 Australia plant species were in the range of 0.75 – 0.82 after receiving stormwater with 0.393 ± 0.008 mg L^{-1} NO_x-N and 0.260 ± 0.017 mg L^{-1} TP. However, some plants in the present study showed physiological stress when watered with N10 solution. An example was *Bhe. paniculata*, which showed lower F_v/F_m readings compared to the control plants (Figure 2C). Photochemical quenching parameters (F_v/F_m, F_v'/F_m', Φ_{PSII}, and qP) all decreased significantly in *Bhe. paniculata* N10 plants, whereas non-photochemical quenching (NPQ) increased (Figures 2C, 6B, 8B, 10B, and 12B). *Bhe. paniculata* plants are secondary and swamp forest plants, and from these data, planting this species for treating roof runoff would be more ideal than road surface runoff which may have higher concentration of nutrient pollutants as the N10 solution showed a detrimental effect on photochemical efficiency. The same could be true for *Syz. myrtifolium* plants, which showed variable F_v/F_m readings when watered with N10 solution (Figure 4D). For *Dip. kerrii* plants, F_v'/F_m', Φ_{PSII}, and qP were more sensitive indicators which showed decreased efficiency of PSII over time in the light-adapted state (Figure 6E, 8E, and 10E). In the same way, non-photochemical quenching

(NPQ) readings increased significantly (Figure 12E). It could be that the *Dip. kerrii* saplings were adapted to the shaded understory conditions in their natural habitat, as also observed in other Dipterocarpaceae species (Barker et al., 2006; Rana et al., 2009). The Native Plant Nursery conditions where the plants were grown had higher light levels that could pose as a stress factor to the plants. However, it is important to note that most bioretention systems would be situated in open areas where stormwater runoff will be pre-treated before joining the major drainage systems. Such open areas include park spaces, carparks, roadsides, for instance. Thus the conditions in the Native Plant Nursery where the plants were grown were a good test-bed for the suitability of the plants to withstand such high-light growth conditions. In *Bhe. robusta* plants, the chlorophyll fluorescence parameters were not consistent; F_v/F_m, F_v'/F_m', and Φ_{PSII} did not show any significant changes, but qP showed a significant decrease comparing week 1 and week 7 (Figures 2D, 6C, 8C, and 10C). This could indicate that for this species, qP was the most sensitive indicator for plant stress response. All *Che. speciosus* plants showed increased NPQ, indicating an increase in processes that protected the leaves against light-induced damage (Figure 12D) (Maxwell and Johnson, 2000; Gorbe and Calatayud, 2012). However, *Che. speciosus* plants are forest edge plants which should be adapted to high light conditions, thus this increase in non-photochemical quenching might be a result of the acclimatization to the light conditions at the Native Plant Nursery, with possibly lower light level. Finally, *Tristel. australasiae* control plants showed higher NPQ than N10 plants only in week 3 (Figure 13C), which might be due to the on-going adaptation of the

plants to the light conditions in the Native Plant Nursery during the acclimatization.

Most plants showed no significant changes in SPAD readings, which have been shown to be strongly correlated to chlorophyll concentrations (Table 4). Changes in SPAD readings could be related to changes in chlorophyll concentrations and the greenness of the leaves (correlation coefficient ranging from 0.83 – 0.99 for the plants in this study, Figure A-2 – A-6). The wavelengths of light used in the SPAD machine to calculate the SPAD values were based on absorbance data from destructive chlorophyll extraction (Godoy, 2002), making SPAD readings proportional to the chlorophyll concentration in leaves (Argenta et al., 2001). Zhao et al. (2005) used SPAD readings as an indicator of leaf chlorophyll concentrations, and found that leaf chlorophyll concentrations based on SPAD readings were positively correlated to leaf nitrogen concentrations ($r^2 = 0.66 – 0.88$***). When the SPAD readings in this study showed no significant changes, this was an indication that these plants were not under stress from the nutrients added, as some plants showed lower SPAD and chlorophyll concentrations with nitrogen treatment. For example, it was shown that chlorophyll concentrations of *Holcus lanatus* (Poaceae) decreased by 25% when the grass was subjected to 200% Hoagland nutrient solution (Scheirs and De Bruyn, 2004). *Cunninghamia lanceolata* and *Olea europapaea* plants have also been documented to show a decrease in chlorophyll concentrations when nitrogen was added in supraoptimal doses (48 gN m^{-2} and 200ppm N respectively) (Liao et al., 2010; Fernández-Escobar et al., 2014). Both control and N10 *Cri. asiaticum*, *Cal. longifolia*, *Che. speciosus*, *Pip. sarmentosum*, and *P.*

serratilofia plants showed decreasing SPAD readings from week 1 to 7 (Figures 14E, 14F, 14H, 15H and 16A), indicating that the decrease in leaf greenness, and hence chlorophyll concentration, was not due to the nutrient addition but to other factors. One possibility was that the plants which were purchased from the supplier, where plants were grown in a lower light condition compared to the Native Plant Nursery, and the higher light intensity during the growth period in the Native Plant Nursery caused a reduction in chlorophyll concentration and hence SPAD reading. This same observation was shown in a variety of up to 86 different Neotropical forest plant species during high light acclimatization, as well as *Nicotiana tabacum* (700–800 μmol photons m^{-2} s^{-1}), *Parthenium argentatum* (1250 μmol m^{-2} s^{-1}), and *Arabidopsis thaliana* (1600 μmol m^{-2} s^{-1}), for example (Ballottari et al., 2007; Matsubara et al., 2009; Biswal et al., 2012; Turan et al., 2014). In *Bhe. robusta*, *Gar. tubifera*, *Lit. sundaicus*, and *Tristel. australasiae* N10 plants, SPAD readings were consistently high possibly due to the effect of the increased nitrogen load (Figures 14D, 15C, 15E, and 17). Nitrogen is a major building block for chlorophyll synthesis and an important macronutrient (Taiz and Zeiger, 2002), therefore an increase in nitrogen might have helped the plants to maintain the high SPAD readings. Nitrogen intensifies the green colour of the leaves and promotes photosynthesis because nitrogen increases the amounts of chlorophylls and photosynthetic enzymes (Taiz and Zeiger, 2002; Aroiee and Omidbaigi, 2004; Sedano-Castro et al., 2011). However, in the control plants of *Bhe. robusta*, *Gar. tubifera*, *Lit. sundaicus*, and *Tristel. australasiae*, the SPAD readings decreased and this might be due to the acclimatization to the high light conditions, as explained for *Cri. asiaticum*,

Cal. longifolia, *Che. speciosus*, *Pip. sarmentosum*, and *Pre. serratifolia* plants above. It was unlikely that the lower chlorophyll concentration was a result of nitrogen deficiency as compost was added to the growth medium to ensure the healthy growth of the plants, even when the pots were not loaded with nutrients from the N10 solution.

In forest ecosystems, nitrogen is generally the limiting resource for tree growth (Bobbink et al., 2003; Magnani et al., 2007). However, not all trees benefit from high nitrogen load as it may change soil pH and the availability of other nutrients needed for plant growth such as phosphorus and magnesium, resulting in nutrient imbalance, plant stress, and chlorosis (Nakaji et al., 2001). However, the growth medium in all pots in this study had neutral pH (Figures 30, 31, 32, and 36), which suggested that the nutrient load did not affect the pH of the leachate. Furthermore, if the nutrient addition did not significantly alter plant growth in control and N10 plants, it could be suggested that plant growth was not limited by nutrients and the N10 solution contained low nitrogen and phosphorus concentrations. The growth parameters monitored in this study were leaf length, leaf production, and the total number of leaves (only for some species). Plant height was not measured as it was observed that the plants showed very insignificant increases in height over the 7 weeks of non-destructive growth and experiments, and some species were too tall for frequent height determination. Leaf length of the mature leaves did not change significantly for most plants, except *Cal. longifolia*, *Dip. kerrii*, and *Tar. odorata* (Figure 18). The decrease in leaf length in mature leaves of *Cal. longifolia* and *Dip. kerrii* plants could be because of leaf burns (Figure 21) as the edges of the leaves were burnt by high light, resulting in the leaf length

becoming slightly shorter. However for *Tar. odorata*, the leaf length reduction in mature leaves could be due to the lower light conditions of the Native Plant Nursery compared to where the plants were purchased from. Although the chlorophyll fluorescence data did not show signs of stress (Figure 4H, 7B, 9B, 11B, and 13B) and the SPAD readings did not increase (Figure 16H), it has been documented before in *Parthenium argentatum* plants that in low light conditions (100 μmol m^{-2} s^{-1}) compared to high light conditions (1250 μmol m^{-2} s^{-1}), overall leaf area is smaller in low light conditions (Turan et al., 2014).

From the results, the total number of leaves (Figure 19) of *Bar. asiatica*, *Cri. asiaticum*, *Dip. kerrii*, *Ela. tapos*, *Pla. obovata*, and *T. austalasiae* plants showed that the plants maintained leaf production and leaf senescence rates at equilibrium. This was further confirmed by the new leaf growth, which showed that *Bar. asiatica*, *Cri. asiaticum*, *Dip. kerrii*, *Ela. tapos*, *Pla. obovata*, and *Tristel. australasiae* plants did not show new leaf production (Figure 20) and also did not show any changes in total number of leaves (Figure 19). Plants abscise their leaves for a number of reasons, and the stress of repotting the plants and acclimatization might have caused leaf senescence to escalate, or the plants might abscise their leaves as a water saving strategy (Kozlowski and Pallardy, 2002; Rouhi et al., 2007; Fini et al., 2013) if the roots had not yet established due some damage during the repotting process. The roots of a plant function to take up water, and damaged roots or root loss would impair the ability of the plant to take up sufficient water, hence resulting in water stress symptoms. For example, *Jatropha curcas* withstands water stress by selective abscission of leaves as a water-saving mechanism (Fini et al., 2013), and *Prunus lycioides* only kept some

leaves while shedding the rest as a water stress coping strategy (Rouhi et al., 2007). However, when the total number of leaves did not decrease even though the plants did not produce new leaves, it indicated that leaf senescence or abscission as a result of stress or tissue damage was not accelerated. The total number of leaves for *Ste. macrophylla* plants increased in both control and N10 plants (Figure 19), and new leaf production was also recorded for both control and N10 plants (Figure 20), showing that the N10 solution did not improve leaf production, and all the plants were simply growing well from week 4 – 7. The total number of leaves of *Bhe. robusta*, *Cal. longifolia*, *Pip. sarmentosum*, and *Sch. elliptica* plants did not increase (Figure 19) even though leaf production was evident (Figure 20), indicating that there, most likely, was some shedding of leaves due to the stress of repotting.

Cal. longifolia, *Lit. sundaicus*, *Sch. elliptica*, and *Syz. leucoxylon* showed an increase in leaf production on the tagged branch when irrigated with the N10 solution compared to the control plants (Figure 20), showing that the plants were not only healthy when irrigated with the N10 solution (see also chlorophyll fluorescence results), they were able to assimilate the added nutrients and increase biomass production. An increase in nitrogen fertilization could influence an increase in plant biomass. In a previous study on potato crop, increased nitrogen fertilization showed increased leaf production and foliage mass, and potato leaf mass was mainly affected by nitrogen fertilizer amount (Ruza et al., 2013). When treated with 12 gNm^{-2}, *Cunninghamia lanceolata* plants were reported to show an increase in shoot, root, and whole plant biomass (Liao et al., 2010). Denman et al. (2007) found that *Eucalyptus polyanthemos*, *Lophostemon confertus* and *Platanus orientalis* trees in

Australia showed increased growth when receiving stormwater presumably because of the added nutrients, and the same could be true for *Cal. longifolia*, *Lit. sundaicus*, *Sch. elliptica*, and *Syz. leucoxylon* plants as these species also showed an increase in biomass production during the irrigation with N10 solution in this study. Although increased nitrogen fertilization can increase plant biomass, supraoptimal concentrations of nitrogen could have the opposite effect and result in reduced plant biomass. *Cunninghamia lanceolata* plants, which were treated with 48 gN m^{-2}, showed lower shoot, root, and whole plant biomass compared to plants treated with for 12 gNm^{-2} (Liao et al., 2010). *Bhe. paniculata* and *Tristan. whiteana* N10 plants showed slower leaf production compared to control plants (Figure 20), indicating that the plants were possibly stressed by the increase in nutrient load. As most tropical forest plants grow on relatively nutrient poor soils (Baker et al., 2003), the N10 solution would pose as a fertilizer for these forest species, which is different from the conditions in their natural habitat. These growth parameter data for *Bhe. paniculata* substantiated the earlier results on chlorophyll fluorescence (Figures 6B, 8B, 10B, and 12B), which showed that the plants showed signs of stress when irrigated with N10 solution compared to the control plants. Although it might seem unlikely that added nutrients could slow the growth of the plants, this has also been documented in olive plants, where high concentration of nitrogen (200 ppm) decreased the growth rate of olive plants (Fernández-Escobar et al., 2014). Although the N10 solution was not as concentrated as other studies, the amount of nitrogen added might have been greater than that in the typical nutrient poor soils in tropical forests that the plants were naturally adapted to. *Bac. minor*, *Cle. sumatranus*, *Che. speciosus*,

Gar. tubifera, *Hop. ferrea*, *Pae. foetida*, *Pre. serratifolia*, *Syz. myrtifolium*, *Tal. tiliaceum*, and *Tar. odorata* plants showed insignificant new leaf growth and thus might be naturally relatively slower growing plants compared to other species.

It is important to understand that the visual quality of the plants during the growth period as the plant establish themselves in the bioretention systems also have a role to play in terms of adding aesthetic value to the surroundings. This aesthetic appeal could not be documented in terms of chlorophyll fluorescence and difficult to visualize in terms of chlorophyll concentration. The visual quality score was in terms of the entire plant, and it was slightly different from the SPAD and chlorophyll concentration results which would monitor the mature leaves of the plants. Thus the visual quality scores gave an idea of how healthy and/or appealing the plants look overall, even when grown in the prevailing (harsh) conditions. Overall, all species showed scores very close to the target of 15 (Figure 21). Even though there were some leaves that were wilted, yellowed, or burnt, those were mainly the older leaves and it was inevitable and natural that the older leaves would yellow during senescence as chlorophylls were degraded (Hörtensteiner and Kräutler, 2011). When the plants are grown under constant environmental conditions, senescence occurs in plants naturally as a response to aging, and should be relatively constant and predictable (Hensel et al., 1993). The photosynthetic apparatus are the major source of nitrogen, and when nitrogen is the main limiting factor for growth, the degradation of soluble as well as membrane-bound photosynthetic protein constituents and photosynthetic pigments for its subsequent recycling is a hallmark of leaf senescence (Hörtensteiner and

Kräutler, 2011). This degradation of chlorophylls in senescing leaves would cause the leaves to yellow. At the same time, it is usually the older leaves which would respond to stress quicker than younger leaves (Weaver et al., 1998), and certain abiotic stress factors and hormones such as drought, detachment, absicic acid (ABA) or ethylene can hasten the yellowing and senescence process (Malik, 1987; Becker and Apel, 1993; Jing et al., 2005; Kacprzyk et al., 2011). Aesthetic appeal of the plants are a part of the suitability for planting in bioretention systems as this will encourage the general public to appreciate and value such green systems that protect their waterways. Furthermore, the general public will become the stakeholders of systems planted in their community, and having an aesthetically pleasing garden will encourage ownership over the maintenance and care of the system.

5.2 Plants and the effect on soil

Soil moisture is an important aspect to consider for the maintenance of plant health. The watering regime of 3 – 4 days was close to the rain frequency of an average of 3 rain days per week that Singapore naturally receives throughout the year, calculated based on a 27 year period (NEA, 2009). It is important to understand how the plants would affect soil moisture as studies have shown that nitrate tends to be washed out of the filter media in significantly higher amounts (effluent concentration 3.4 to 6 times higher than the influent concentration) upon re-wetting following a dry period (5 – 20 antecedent days) compared to during wet periods (effluent concentration converged to influent concentration with 5 or less antecedent dry days) (Hatt et al., 2007b; Cho et al., 2009). The leaching of nitrate could be attributed to

the accumulation of nitrate through the nitrification process during dry periods (Cho et al., 2009). In this regard, it would be helpful to know which species would maintain high soil water content even during dry periods. *Bac. minor* was the only species to show a significantly higher moisture content compared to non-vegetated pots (Figure 22A), and this might be important to prevent nitrate leaching when the number of antecedent dry days is high. Plants may be water-stressed if the number of dry days is high; however, a study has shown that even 5 antecedent dry days could result in the effluent nitrate concentrations exceeding influent concentrations due to nitrification, and this increased with the number of antecedent dry days (Cho et al., 2011). Therefore, the plants may have a positive effect to prevent such leaching by increasing the water content of the growth/filter media, without becoming too water-stressed. Pots planted with *Bhe. robusta*, *Cal. longifolia*, and *Dip. kerrii* maintained a constant soil moisture content even though the soil was decreasing in moisture throughout the weeks of experiments (Figures 22D and E and 23C). This indicated that the presence of the plants helped to keep the soil moist, compared to the filter media in non-vegetated pots. However, the majority of the plants studied either showed no change in soil moisture or a decrease in soil moisture (range of 6.2% – 27.3%) compared to non-vegetated pots (Figures 22B, C, F, 23A, B, D–H, 24A–H, and 25). On the other hand, denitrification rates have been reported to increase with soil moisture (Klein and Logtestijn, 1994; Smith et al., 1998) as the water content determines the oxygen transfer rate from the atmosphere to the sites where biological degradation occurs (Smith et al., 1998). It has also been previously suggested that the minimum volumetric water content for denitrification should be 40%

in loam soil (Klein and Logtestijn, 1994), and this would aid in nitrate removal through anaerobic denitrifying bacteria. However, when soil moisture is so high, it may give rise to soilbourne diseases in the roots (Abawi and Widmer, 2000). Wet, compact soils may also increase plant mortality due to root rot (Rhoades et al., 2003) and in the present study, soil moisture never reached 40%. Hypoxia or anoxia is damaging to the roots of plants and water-logging in the root environment can deprive the roots of the much needed oxygen (Shi et al., 2007; Kläring and Zude, 2009). Thus, in this study, the soil moisture content was always kept at a level below 30%, which could sustain plant health instead of creating an anoxic environment for denitrification.

Plants are also important for bioretention systems as such systems are prone to clogging due to the high loading rates of stormwater runoff into a small area as bioretention systems typically make up only a small percentage of the total catchment size (Le Coustumer et al., 2012). As such, the maintenance of the permeability of the filter media over time is important for the proper functioning of the bioretention systems in the long term. Plants have root systems which can create and maintain pores and paths in the filter media for water flow. The flow rate of water through the growth/filter medium was studied as a preliminary indicator of the permeability of the filter media with and without the presence of the plants. This study might have been conducted over too short a duration to show significant changes in the flow rate over time. However, in just 7 weeks, the large pots of non-vegetated soil started to show evidence of slowing flow rate due to compaction compared to vegetated pots (Figure 37). This observation was similar to a study conducted in Australia that showed how the barren system started to clog by a factor of

3.6 over a 72 week period, with the first observation of significantly reduced hydraulic conductivity only at 39 weeks (Le Coustumer et al., 2012) although on a different time scale. In this present study, the total exfiltration was still completed within an hour, and this result showed how quickly the filter medium could become compact without the presence of plants, and how important it is for bioretention systems to be vegetated.

5.3 Nitrate and phosphate removal

Studies have shown that vegetation has a substantial effect on improving nitrate removal and variation in nitrate removal could be largely dependent on plant species (Henderson et al., 2007; Bratières et al., 2008; Lucas and Greenway, 2008; Read et al., 2008; Read et al., 2010). When comparing pollutant removal standardized for plant size, Read et al. (2008) reported a 18–50-fold variation in effluent concentrations of total P and N, and a 150-fold variation in NO_x among the 20 Australia plant species in their study. A similar trend was observed in the present study, in which nitrate removal per plant mass showed a 20-fold variation from one plant species to another plant species. Furthermore, Read et al. (2008) reported NO_x species removal in planted treatments to be 21% on average, whereas planted treatments in this study showed an average of 35% removal for NO_3. In another Australia study, 240L mesocosms planted with *Pennisetum alopecurioides*, *Dianella brevipedunculata*, *Banksia integrefolia*, and *Callistemon pachyphyllus* showed 47% nitrogen oxides removal attributed to plant uptake (Lucas and Greenway, 2008). In this present study, the nitrate removal ranged from an average of 19% – 56% for planted pots, compared to

an average of 26% – 46% in barren pots. In general, all plants in this present pot study showed nitrate removal from the soil for at least one week of the irrigation, except *Dip. kerrii* plants (Figures 26 – 29). Some species required a few weeks before nitrate removal from the soil was enhanced by the presence of the plants, and this might indicate that these plants needed a longer time to become acclimatized to the growth conditions, before they exhibited the nutrient removal. Such species included *Bhe. paniculata*, *Ela. tapos*, *Gar. tubifera*, *Syz. leucoxylon*, and *Tal. tiliaceum* (Figures 26C, 27B, 27C, 28D, and 28F). On occasions when the nitrate removal of vegetated pots was lower than non-vegetated pots, the soil was probably removing more nitrate compared to the vegetated pots. One reason for this increase in nitrate concentration in the effluent when the soil was vegetated (lower nitrate removed) could be that the plants were producing a high amount of root exudates (Kloepper et al., 1989; Gamalero et al., 2002; Glick, 2003; Gamalero et al., 2004; Glick, 2004; 2010). These root exudates are important for attracting specific groups of microorganisms which have symbiotic relationships with the plants. These symbiotic microorganisms would proliferate and colonize the roots of the plants, positively affecting plant growth (Kloepper et al., 1989; Glick et al., 1995), enhancing root development (Gamalero et al., 2002; Gamalero et al., 2004), and increasing the tolerance of plants to various environmental stresses (Glick, 2004). In turn, larger, healthier plants are better able to phytoremediate contaminants and grow in such bioretention systems (Glick, 2003; 2010). Although nitrate removal was variable and occasionally high in the presence of different species, phosphate removal was insignificant throughout the experiments for all plant species (Figures 26 – 29). Adsorption to soil particles

is considered as one of the predominant phosphorus removal mechanisms in bioretention systems and accretion into the sediments is a long-term phosphorus removal process (Lai and Lam, 2009; Li and Huang, 2013). In this study, the phosphorus load was low and the probable high phosphorus adsorption by the growth medium might have contributed to the lack of significant difference between vegetated and non-vegetated pots. Even though accretion into the sediments may be considered a long-term phosphorus removal process, vegetation has been shown to be an important feature in regulating the phosphorus holding capacity of soil (Liu et al., 2013; Zhang et al., 2013), and plants may act as a phosphorus sink when it is accumulated in the biomass and the plants are harvested or cleared regularly.

The presence of the plants did not influence significant changes in the conductivity of the leachates, except for pots planted with *Bar. asiatica*, *Cle. sumatranus*, *Gar. tubifera*, *Lit. sundaicus*, *Pip. sarmentosum*, *Syz. myrtifolium*, *Tal. tiliaceum*, *Tar. odorata*, and *Tristel. australasiae* plants. For *Tal. tiliaceum* plants, the conductivity of the leachate was significantly reduced (Figure 35), corresponding to the nitrate removal (Figure 28F), and likely reflected a decrease in the number of ions (anions) due to enhanced removal by the presences of the plants. Although *Bar. asiatica*, *Cle. sumatranus*, *Gar. tubifera*, *Lit. sundaicus*, *Pip. sarmentosum*, *Syz. myrtifolium*, *Tar. odorata*, and *Tristel. australasiae* plants did not show any significant improvement in the nitrate and phosphate removal (Section 4.8), compared to the non-vegetated pots, the significant reduction in conductivity in vegetated pots suggested that ions other than nitrate and phosphate were removed by the plants. However, as

the anion species of this study were nitrate and phosphate, the other ions in the effluent were not studied (see Table A-1 – A-5).

5.4 Plant biomass responses

The dry weight of the reproductive organs was presented as part of the total dry weight of the plants (Figure 38) as the dry weight of reproductive organs (if any) was very small. Dry weight was only determined at the end of the non-destructive experiments when the plants were harvested. Thus, there was no initial dry weight recorded and the differences in dry weight recorded for various species at the time of harvest might have been manifested at the start of the non-destructive experiments, even though the plants were chosen to be roughly the same size by visual estimation. The SLA of *Bac. minor* N10 plants was significantly higher than that of control plants (Figure 39), even though the TSP was significantly lower (Figure 40) and no significant differences could be found for chlorophyll concentration (Figure 14A), TKN (Figure 41), and TP (Figure 42). Although *Bac. minor* plants only showed nitrate removal during one week of the experiments (Figure 26A), it was evident that the added nutrients resulted in dry matter gained as observed from the SLA data, showing this species would be a good nutrient sink. It is beneficial for plants to convert and accumulate the nutrients into aboveground biomass as this means it can be easily harvested or cleared from the system as a permanent nutrient removal method without having to harvest the entire plant and re-vegetate the system. *Cri. asiaticum* N10 plants showed significantly lower SLA compared to control plants (Figure 39). The reason for this is unknown as the chlorophyll concentration (Figure 14E), TSP

(Figure 40), TKN (Figure 41), and TP (Figure 42) were all not significantly different between control and N10 plants leaves. This difference in SLA might have been due to an adaptation of the species to require less mass per leaf surface area in the Native Plant Nursery due to a change in light conditions compared to the nursery where the supplier had grown them.

Although there is much evidence for the effectiveness of plants to improve nutrient removal in bioretention systems, there have also been studies reporting no significant difference between vegetated and non-vegetated systems (Balizon et al., 2002; Calheiros et al., 2007). In the study by Calheiros et al. (2007), systems planted with *Canna indica*, *Typha latifolia*, *Phragmites australis*, *Stenotaphrum secundatum*, and *Iris pseudacorus* showed effluent concentrations of 31 mg NO_3 L^{-1} and 0.37 – 0.56 mg TP L^{-1}, very similar to the unplanted control of 32 mg NO_3 L^{-1} and 0.4 mg TP L^{-1}. Although nutrient removal might not be detected in all the leachates analysed in the present study, the added nutrients generally affected the growth of the plants positively (Section 4.5) and the plants did not show physiological stress (Section 4.2). Thus this might improve the nutrient removal of bioretention systems in a more indirect manner. For example, leaves of *Cal. longifolia* and *Ela. tapos* N10 plants showed significantly higher TKN compared to the leaves of control plants (Figure 41) even though the plant did not improve nitrate removal (Figure 26E) or only enhanced nitrate removal for 1 week (Figure 27B). This showed that even when nutrient removal could not be detected from leachate analysis or nutrient removal was poor, the plants were assimilating these nutrients into their biomass, in terms of TKN for nitrogen, for example. In a study by Tripathi et al. (2014) on five tropical dry forest

trees, excess nitrogen supplied under fertilized conditions (120kg N ha^{-1} treatment) led to accumulation of nitrogen in the leaves. Although the current study did not supply nutrient load to the same extent, the *Cal. longifolia* and *Ela. tapos* plants showed accumulation of nitrogen in terms of TKN in the leaves as well. These plants would also be effective in taking up these nutrients to assimilate them into dry matter. TKN was also significantly higher in the reproductive organs of *Tar. odorata* N10 plants (Figure 41), showing that the nitrogen might not only be accumulated in the leaves but in the reproductive organs as well. Also, even though phosphate seemed to be removed mainly by adsorption by the filter medium, as phosphate removal showed no significant differences in vegetated and non-vegetated pots for all species (Figures 26 – 29), TP was significantly higher in the leaves of *Gar. tubifera*, *Lit. sundaicus*, and *Syz. leucoxylon* N10 plants (Figure 42). This showed that these species took up phosphate, and the leaves could be easily harvested and removed from the system through pruning or clearing of leaf litter. Unfortunately, there were some plant species which showed accumulation of TP in the roots. These species included *Cal. longifolia* and *Pre. serratifolia* (Figure 42). The data obtained indicated that *Cal. longifolia* plants accumulate TKN in the leaves but TP in the roots. This species can still be considered suitable for planting in bioretention systems as TKN accumulation is important and the roots of the plant can still contribute to the maintenance of the porosity of the filter medium. *Pre. serratifolia* plants, like those of *Cal. longifolia*, showed TP storage in the roots. These plants can be planted in a bioretention system together with those which accumulate TP in

the aboveground biomass to ensure that phosphorus will be effectively removed from the system.

From the results, *Bac. minor* and *Pae. foetida* control plants showed higher TSP concentration compared to N10 plants (Figure 40). Determination of TSP was only conducted for the leaves as plant leaf tissues have a high content of soluble proteins compared to the roots and stems. About 50% of TSP in leaves are ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme catalysing carbon dioxide fixation in plants (Taiz and Zeiger, 2002; Feller et al., 2008). Although chlorophyll levels in these plants did not decrease (Figures 14A and 15F), the amount of TSP (and possibly Rubisco) in the leaves might have been reduced as a sign of stress from the excess nutrients. In *Bar. asiatica* N10 plants, TKN levels in the leaves were significantly lower compared to control plants (Figure 41). It has previously been shown that nitrogen content in entire olive plants was reduced when fertilized with higher concentration of nitrogen (200 ppm) (Fernández-Escobar et al., 2014), and the same might be true for *Bac. minor*, *Bar. asiatica*, and *Pae. foetida* plants. Although the concentration of nitrate added in this study was only 10ppm, the growth medium used had 20% compost by weight, which might have caused the nitrogen content of the medium to be higher than the levels suitable for *Bac. minor*, *Bar. asiatica*, and *Pae. foetida* plants. There were plants which had significantly reduced TP when irrigated with N10 solution. Such species were *Bar. asiatica* (roots), and *Dip. kerrii* and *Ela. tapos* (stems) (Figure 42). In *Bar. asiatica* plants, levels of TKN in the leaves and TP in the roots were significantly reduced when given the added nutrients; this species would be recommended for bioretention systems that do not treat

stormwater runoff, but roof runoff instead, as it typically has lower nutrient content. For *Dip. kerrii* plants, it was interesting to note that although chlorophyll fluorescence data showed the plants were slightly stressed possibly due to the light conditions of the nursery compared to the plant's natural adaptation to shaded understorey conditions (Section 4.2) (Barker et al., 2006; Rana et al., 2009), the only destructive parameter which showed a possible sign of stress was the significantly lower TP in the stems of N10 plants (Figure 42). Dipterocarp seedlings in Malaysia were shown to grow well in full sunlight when provided with enough nutrients (4.8g nitrogen and 2.1g phosphorus in a 4 × 12 cm polybag) (Nussbaum et al., 1995) and it might be possible that the N10 solution and compost added in this experiment provided insufficient nitrogen and phosphorus for *Dip. kerrii* plants to grow well under the full sunlight conditions in the Native Plant Nursery. Lastly, the stems of *Ela. tapos* N10 plants showed significantly reduced TP (Figure 42) whereas *Ela. tapos* N10 plant leaves showed significantly higher TKN (Figure 41). Since the chlorophyll fluorescence results did not indicate any signs of stress, this might just be the plant's unique response to the nutrient supplementation. A study on *Prunus persica* var. *nucipersica* trees showed a similar trend to *Ela. tapos* where increased TKN in the leaves was observed with increased nutrient supplied in the form of compost added, but no difference was observed for other nutrients including phosphorus (Baldi et al., 2014). Nutrient storage and distribution in the plant parts vary among species, and a previous study on nutrient supplementation in the form of compost and plant tissue composition in terms of TKN and TP in *Begonia semperflorens* "Bellavista F1", *Mimulus* "Magic × hybridus", *Salvia splendens* "maestro",

and *Tagete patula* × *erecta* “Zenith Lemon Yellow” also showed marked variation among species as compost concentration increased in the substrate (Grigatti et al., 2007).

5.5 Natural habitat and plant traits in relation to nutrient removal

Generally, the removal and accumulation of nitrate and phosphate by the different species seemed to be extremely species-dependent, and this seemed to be related to their natural habitats. The data indicated that the plants that grow naturally in the forest edge (*Che. speciosus*, *Ela. tapos*, and *Pae. foetida*) seemed to exhibit consistent nutrient removal. It would be highly beneficial to find out which plant traits would confer greater nutrient removal, thus plant species could be chosen for planting in bioretention systems based on these plant traits, without having to go through the rigor of lengthy scientific experiments. The study by Read et al. (2010) showed that the length of longest root and root soil depth contributed strongly to pollutant removal. However, the present study did not focus on the root length and depth as this was a pot study with plants planted in pots of limited depths. In the case of lined bioretention systems, the plants would have a limited growth area, although not as small as the pots. The size of the pots used was sufficient for the plants to grow healthily, although root growth was restricted; root balls formed and root depth could not be accurately studied (Plate 26 and 27). The roots of most of the plants studied displayed dense, fine root systems, deemed to provide the best nutrient removal performance by a study conducted on plants native to Australia (Read et al., 2010). However, this might not be the case for species that are native to Singapore as they showed high variability in

pollutant removal in this study. The root characteristics described in Section 4.16 displayed no clear patterns for plant habit or natural habitat. Furthermore, root thickness will differ during the plant's life stage as an older, larger plant would have larger root systems compared to a younger, smaller plant of the same species. Nonetheless, a simple regression was conducted to understand how the root thickness of the plants studied affected nitrate and phosphate removal, as well as the flow rate of water through the pots.

The plant and root masses were also studied in relation to nitrate and phosphate removal and flow rate. As the study by Read et al. (2010) was conducted on Australian plants, it is important to know whether the plant traits that affect the effectiveness of plants in bioretention systems would be similar in tropical plants. Surprisingly, strong correlations were found between root and total plant dry mass and nitrate and phosphate removal in trees species (Figure 44), contrary to what was reported in the Australian study like *Juncus amabilis*, *Banksia marginata*, *Correa alba*, *Hibbertia scandens*, and *Kunzea ericoides* (Read et al., 2010). When the correlation was analyzed with the total dry mass of the trees, the linear relationship was even stronger where 89% and 60% of the variation in nitrate and phosphate removal respectively were related to the total dry mass of the trees (Figure 45). This showed the value of planting tree species in bioretention systems as they do have vast potential to remove large amounts of pollutants due to the extensive root systems and biomass. Tree root thickness also showed a statistically significant relationship to nitrate and phosphate removal in trees ($p < 0.05$), suggesting that large trees with thick roots would be beneficial for planting in bioretention systems for their high efficiency in pollutant removal. Furthermore, plants with thick roots

are regarded to be able to create significant macropores in the filter media and are deemed the most effective in protection against filter media clogging (Le Coustumer et al., 2007; Read et al., 2010). Finally, for non-tree species, the total dry mass showed a significant relationship to flow rate ($p < 0.05$) but root thickness was only weakly correlated ($p = 0.073$) to flow rate (Figures 45 and 46). This indicated that for non-tree species, a smaller plant size with thinner roots would result in faster flow rate, opposite to what was observed for tree species. For non-tree species, root dry mass, total plant dry mass, and root thickness were all not related to nitrate or phosphate removed Figures 44, 45, and 46). Non-tree species and tree species in the present study did not show similar correlation trends. These results were different from those reported by Read et al. (2010) where the growth form of the plants (climbers, shrubs, or trees) did not influence the correlation trend of longest root length and root soil depth with the effectiveness nitrate or phosphate removal. Thus for tropical plants, growth form seems to play a role in influencing the nutrient pollutant removal as well as flow rate, compared to the study conducted on Australia species.

In conclusion, the data obtained showed that all plants except *Dip. kerrii* were able to grow well with the added N10 solution. Eleven out of the 25 species studied were able to remove nitrate from the soil. *Tal. tiliaceum* plants exhibited the highest nitrate removal (up to 59%), followed by *Syz. leucoxylon* (up to 52%) and *Pae. foetida* (up to 52%) plants, in contrast to the nitrate removal of barren soil (mean of 34%). Other plants showed lower potential in nitrate removal — *Tristel. australasiae* (up to 42%), *Gar. tubifera* (up to 42%), *Bac. minor* (up to 40%), and *Ela. tapos* (up to 39%). These

results are summarized in Table 3. Both vegetated and barren systems showed the same phosphate removal efficiency of close to 100%.

Conclusions

Bioretention systems are complex systems where plants can play many roles such as enhancing pollutant removal, maintaining the filter media, supporting biodiversity, and adding aesthetic value. In this study, many different species from various natural habitats were studied and although not all showed pollutant removal potential, the plants could play other important roles in the bioretention systems, such as maintaining the filter media, supporting local biodiversity, and adding aesthetic value. The different species studied also showed great variation in responses to the growth environment as plants differed in the chlorophyll fluorescence responses as well as leaf greenness (SPAD) and biomass production. Nutrient accumulation and allocation were also highly variable. From the data obtained, pollutant removal efficiency and flow rate improvement were very species-dependent and variable. However, when compared within two groups, non-tree species and tree species, some conclusions could be made based on plant morphology and size. This study showed that for tree species, total biomass and root thickness might influence nitrate and phosphate removal. For non-tree species, total biomass and root thickness did not influence nitrate and phosphate removal but affected the flow rate. Furthermore, these results are different from those obtained from plants that are native to other countries (e.g. Australia plants), suggesting a regional or climate-dependent relationship between plant traits and bioretention suitability.

However, there are still substantial knowledge gaps that are worthwhile to investigate. A wider range of plants across more different habitats and adapted to different environmental conditions can also be

considered. Additional studies into the root morphology could also be conducted to see how these compare to other studies where root length and depth were attributed to improved pollutant removal. The relationship between the plants and microbial communities as well as the root exudates, and the effectiveness of the associated microbial communities could be examined in terms of pollutant removal effectiveness.

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Appendices

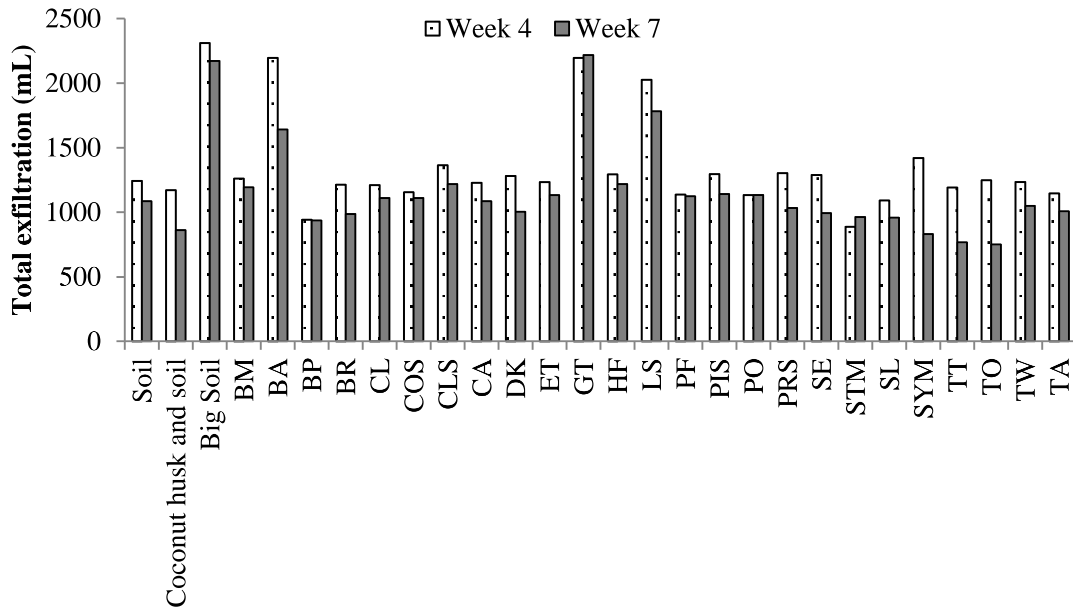

Figure A-1. Exfiltration volumes of effluent from barren soil, coconut husk and barren soil, big pots of barren soil, and pots planted with *Bac. minor* (BM), *Bar. asiatica* (BA), *Bhe. paniculata* (BP), *Bhe. robusta* (BR), *Cri. asiaticum* (CA), *Cal. longifolia* (CL), *Che. speciosus* (COS), *Cle. sumatranus* (CLS), *Dip. kerrii* (DK), *Ela. tapos* (ET), *Gar. tubifera* (GT), *Hop. ferrea* (HF), *Lit. sundaicus* (LS), *Pae. foetida* (PF), *Pip. sarmentosum* (PIS), *Pla. obovata* (PO), *Pre. serratifolia* (PRS), *Sch. elliptica* (SE), *Ste. macrophylla* (STM), *Syz. leucoxylon* (SL), *Syz. myrtifolium* (SYM), *Tal. tiliaceum* (TT), *Tar. odorata* (TO), *Tristan. whiteana* (TW), and *Tristel. australasiae* (TA) at the start of week 4 and the end of week 7.

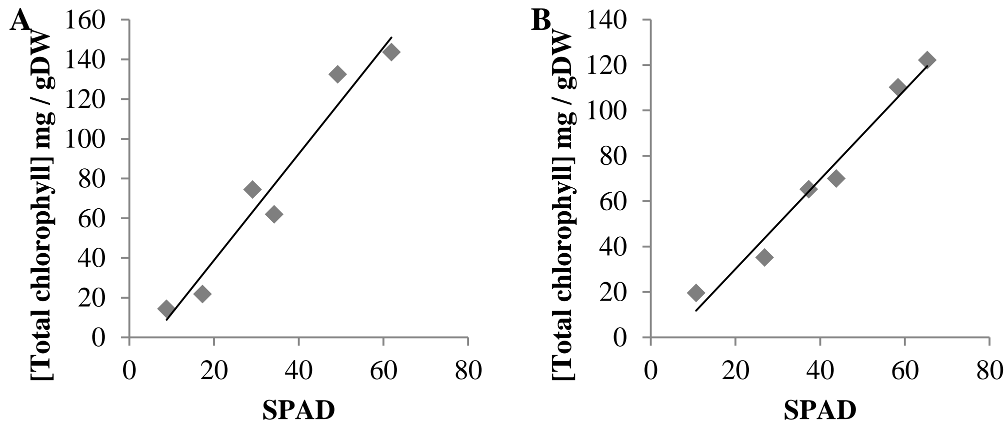

Figure A-2. The scatterplot with simple linear regression of total chlorophyll concentrations verses SPAD for *Bac. minor* (A) and *Bar. asiatica* (B).

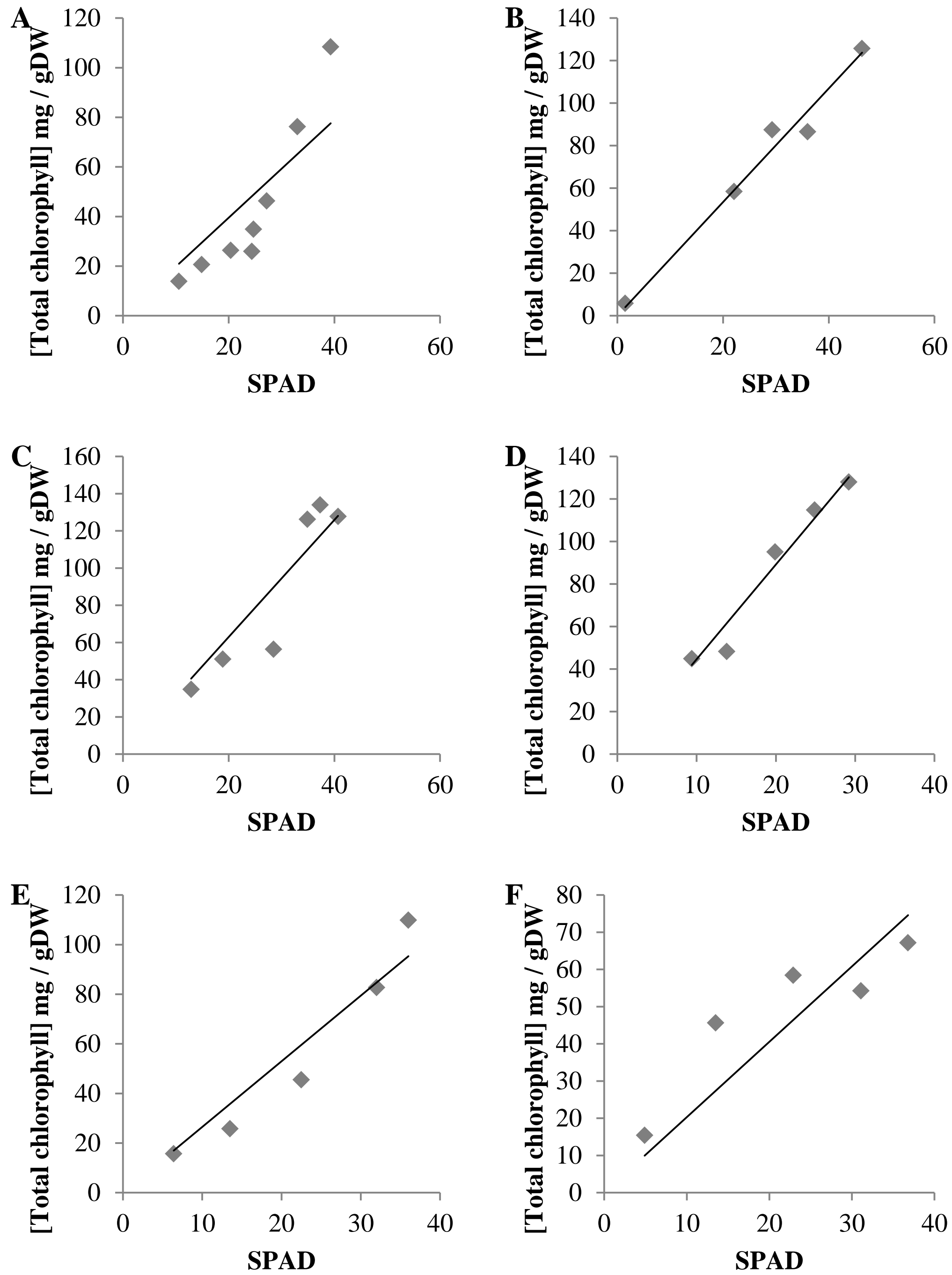

Figure A-3. The scatterplot with simple linear regression of total chlorophyll concentrations verses SPAD for *Bhe. paniculata* (A), *Bhe. robusta* (B), *Cri. asiaticum* (C), *Cal. longifolia* (D), *Che. speciosus* (E), and *Cle. sumatranus* (F).

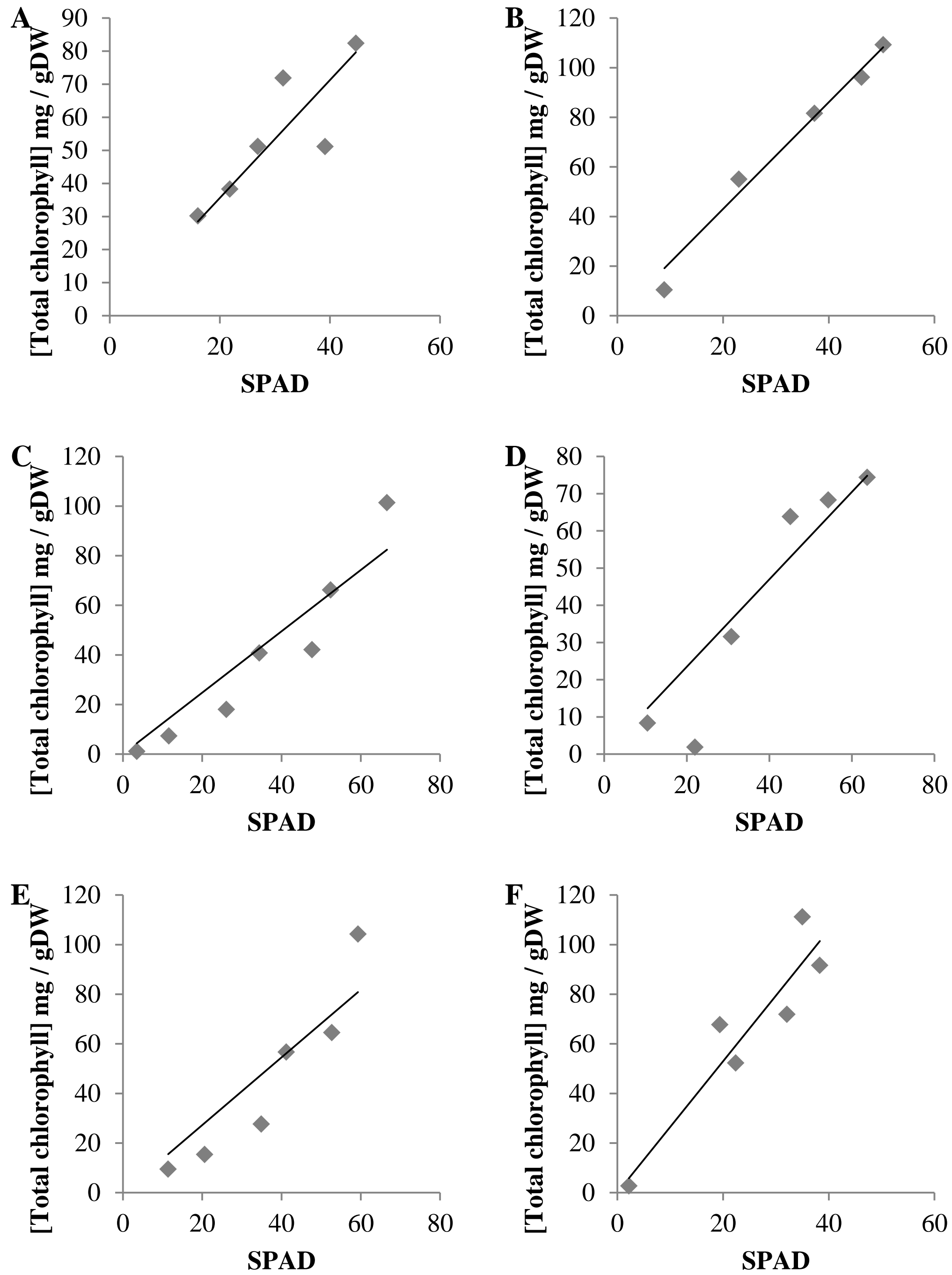

Figure A-4. The scatterplot with simple linear regression of total chlorophyll concentrations verses SPAD for *Dip. kerrii* (A), *Ela. tapos* (B), *Gar. tubifera* (C), *Hop. ferrea* (D), *Lit. sundaicus* (E), and *Pae. foetida* (F).

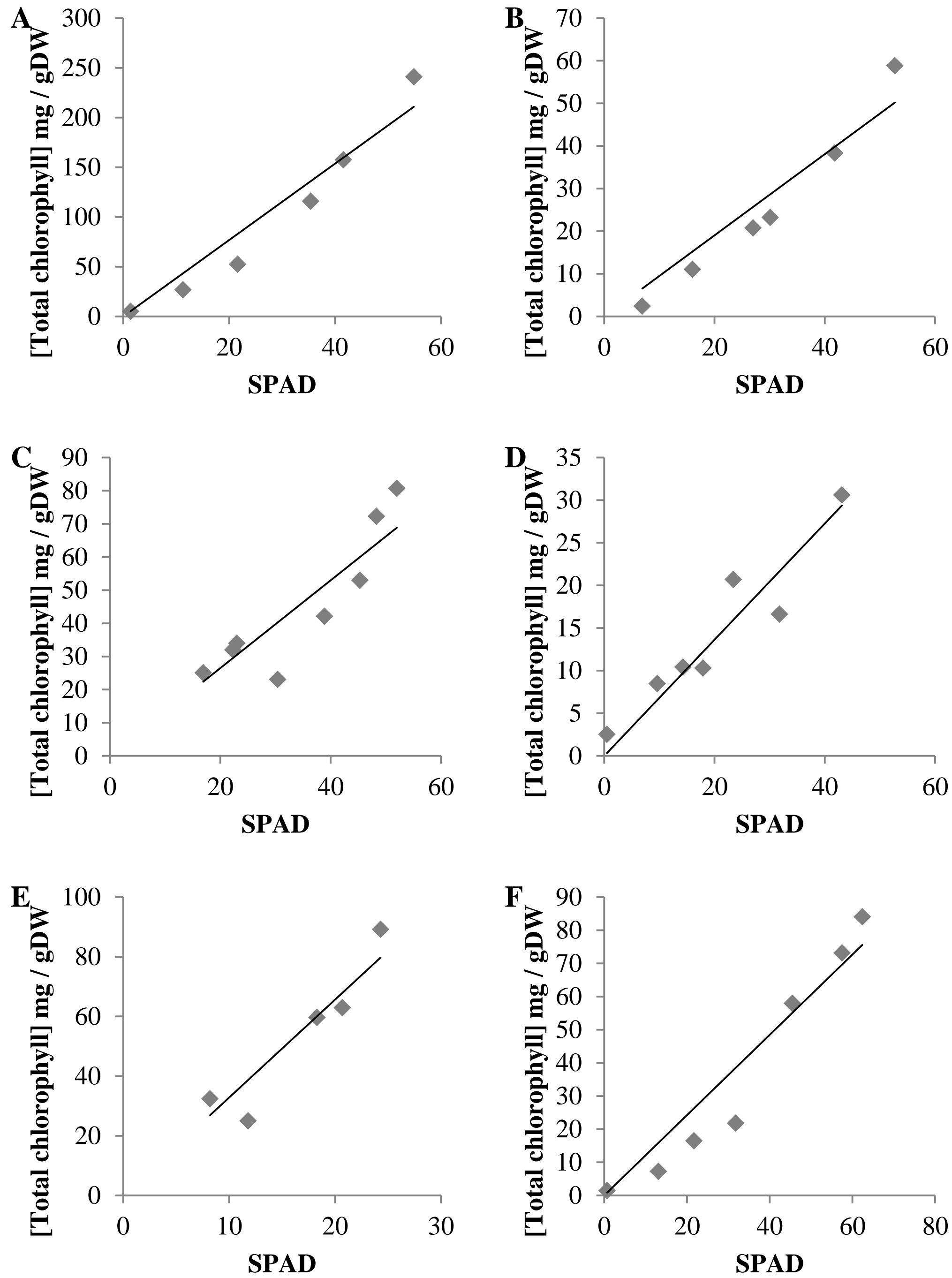

Figure A-5. The scatterplot with simple linear regression of total chlorophyll concentrations verses SPAD for *Pip. sarmentosum* (A), *Pla. obovata* (B), *Pre. serratifolia* (C), *Sch. elliptica* (D), *Ste. macrophylla* (E), and *Syz. leucoxylon* (F).

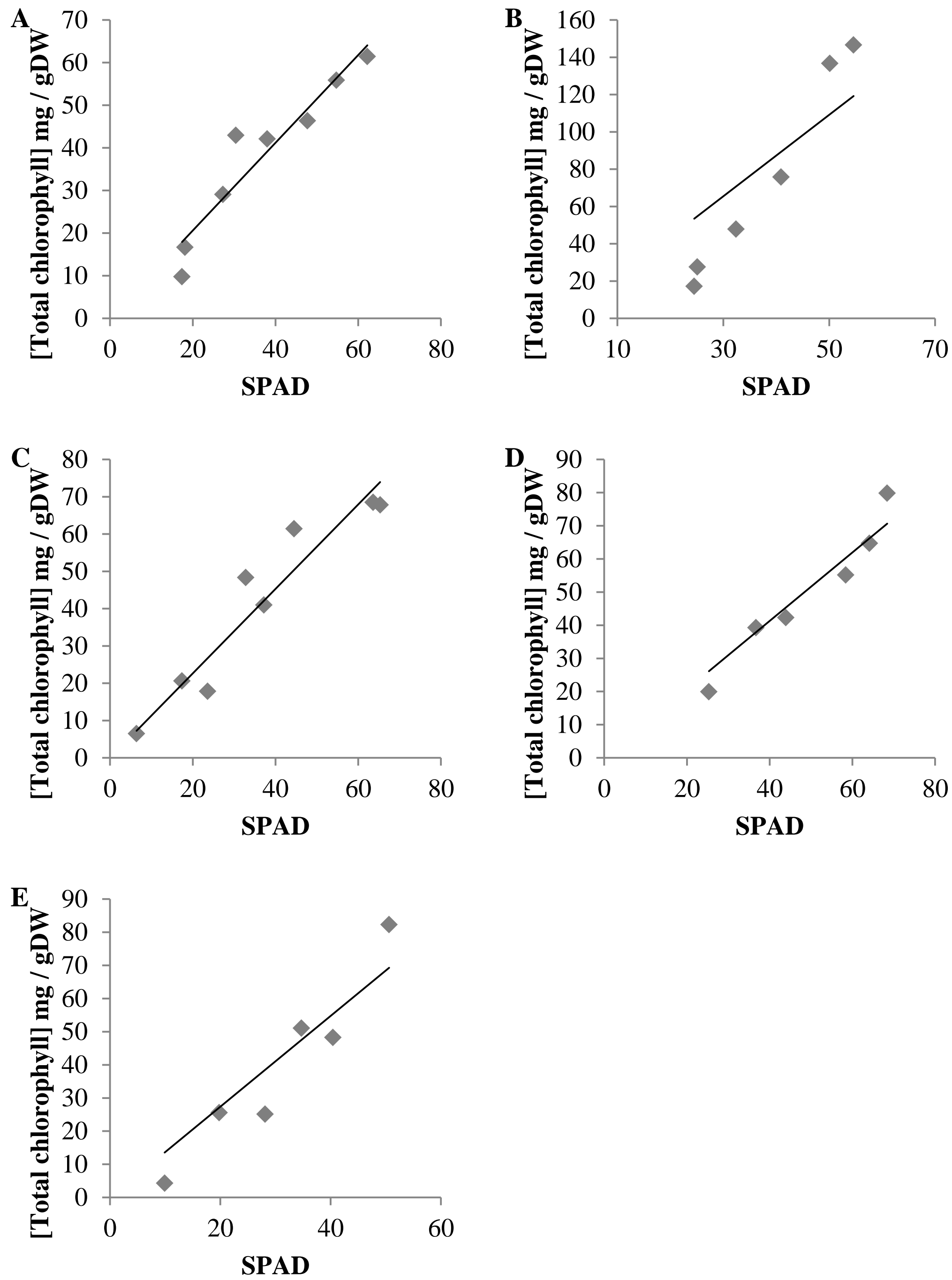

Figure A-6. The scatterplot with simple linear regression of total chlorophyll concentrations verses SPAD for *Syz. myrtifolium* (A), *Tal. tiliaceum* (B), *Tar. odorata* (C), *Tristan. whiteana* (D), and *Tristel. australasiae* (E).

Table A-1. Concentrations of different anions in the leachates of various pots at various weeks presented as mean ± standard error (mg L^{-1}).

		Week 4	Week 5	Week 6	Week 7
Soil	F^-	0.36 ± 0.04	0.32 ± 0.03	0.30 ± 0.03	0.37 ± 0.04
	Cl^-	58.54 ± 13.48	26.16 ± 2.14	24.87 ± 0.59	19.60 ± 1.42
	NO_2^-	0.60 ± 0.09	0.64 ± 0.07	0.56 ± 0.07	0.58 ± 0.08
	Br^-	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.00
	SO_4^{2-}	105.21 ± 12.9	67.88 ± 3.73	55.82 ± 2.45	72.48 ± 7.40
Coconut husk and soil	F^-	0.16 ± 0.08	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
	Cl^-	57.90 ± 2.81	31.82 ± 0.88	41.38 ± 0.84	38.51 ± 0.71
	NO_2^-	0.01 ± 0.01	0.10 ± 0.02	0.04 ± 0.01	0.04 ± 0.02
	Br^-	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.01
	SO_4^{2-}	33.82 ± 0.99	29.43 ± 1.08	49.04 ± 2.83	72.66 ± 2.61
Big soil	F^-	0.28 ± 0.03	0.28 ± 0.02	0.28 ± 0.02	0.30 ± 0.02
	Cl^-	137.13 ± 11.87	60.24 ± 4.37	24.66 ± 2.45	27.55 ± 1.77
	NO_2^-	1.22 ± 0.22	0.53 ± 0.12	1.03 ± 0.27	0.77 ± 0.17
	Br^-	0.20 ± 0.04	0.26 ± 0.05	0.06 ± 0.02	0.10 ± 0.03
	SO_4^{2-}	127.38 ± 16.59	78.39 ± 11.85	59.66 ± 6.96	67.26 ± 5.24
Bac. minor	F^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Cl^-	56.15 ± 12.73	28.70 ± 0.97	26.92 ± 3.04	19.66 ± 2.19
	NO_2^-	0.31 ± 0.16	0.84 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
	Br^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	90.91 ± 25.75	94.44 ± 3.33	90.18 ± 3.25	56.15 ± 1.67
Bar. asiatica	F^-	0.20 ± 0.01	0.27 ± 0.01	0.20 ± 0.01	0.23 ± 0.01
	Cl^-	22.04 ± 4.08	16.85 ± 3.73	18.35 ± 0.62	12.63 ± 2.77
	NO_2^-	1.38 ± 0.22	1.11 ± 0.09	1.02 ± 0.04	1.01 ± 0.14
	Br^-	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
	SO_4^{2-}	139.43 ± 26.95	102.63 ± 12.72	94.66 ± 6.95	85.79 ± 8.31
Bhe. paniculata	F^-	0.25 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.32 ± 0.01
	Cl^-	104.91 ± 5.07	30.37 ± 1.67	21.68 ± 0.68	0.04 ± 0.04
	NO_2^-	0.11 ± 0.01	0.27 ± 0.015	0.28 ± 0.07	0.00 ± 0.00
	Br^-	0.08 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	269.73 ± 9.62	50.24 ± 2.69	43.87 ± 3.66	59.66 ± 3.44

Table A-2. Concentrations of different anions in the leachates of various pots at various weeks presented as mean ± standard error (mg L^{-1}).

		Week 4	Week 5	Week 6	Week 7
Bhe. robusta	F^-	0.47 ± 0.01	0.46 ± 0.02	0.43 ± 0.02	0.44 ± 0.03
	Cl^-	55.53 ± 7.20	32.64 ± 2.35	24.17 ± 0.73	20.29 ± 0.51
	NO_2^-	0.40 ± 0.21	0.67 ± 0.21	0.30 ± 0.17	0.30 ± 0.17
	Br^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	65.09 ± 3.78	48.23 ± 1.74	35.85 ± 0.96	30.62 ± 0.80
Cri. asiaticum	F^-	0.22 ± 0.03	0.24 ± 0.02	0.14 ± 0.01	0.18 ± 0.01
	Cl^-	23.23 ± 0.90	14.63 ± 0.91	14.88 ± 0.81	16.27 ± 0.69
	NO_2^-	0.30 ± 0.13	0.64 ± 0.09	0.06 ± 0.00	0.11 ± 0.05
	Br^-	0.12 ± 0.05	0.13 ± 0.05	0.02 ± 0.01	0.02 ± 0.02
	SO_4^{2-}	38.74 ± 3.42	34.71 ± 2.06	37.85 ± 2.24	54.75 ± 3.18
Cal. longifolia	F^-	0.80 ± 0.03	0.82 ± 0.02	0.77 ± 0.03	0.79 ± 0.04
	Cl^-	30.51 ± 0.88	27.05 ± 0.77	24.31 ± 0.54	20.31 ± 0.56
	NO_2^-	0.28 ± 0.20	0.92 ± 0.25	0.17 ± 0.12	0.00 ± 0.00
	Br^-	0.00 ± 0.00	0.22 ± 0.21	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	70.06 ± 2.99	57.50 ± 3.50	51.06 ± 1.40	42.25 ± 1.55
Che. speciosus	F^-	0.73 ± 0.03	0.68 ± 0.02	0.56 ± 0.06	0.89 ± 0.02
	Cl^-	31.24 ± 1.76	25.44 ± 0.87	15.28 ± 3.33	29.41 ± 3.33
	NO_2^-	0.56 ± 0.20	0.12 ± 0.12	0.00 ± 0.00	0.78 ± 0.28
	Br^-	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
	SO_4^{2-}	72.40 ± 7.97	56.77 ± 2.79	45.73 ± 5.35	61.38 ± 2.77
Cle. sumatranus	F^-	1.37 ± 0.52	0.76 ± 0.03	0.90 ± 0.02	0.88 ± 0.10
	Cl^-	129.02 ± 49.04	9.84 ± 2.70	21.51 ± 0.46	22.31 ± 3.66
	NO_2^-	0.01 ± 0.01	0.11 ± 0.08	0.48 ± 0.13	1.07 ± 0.24
	Br^-	0.47 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	19.39 ± 6.73	32.68 ± 1.75	39.86 ± 1.86	31.92 ± 3.62
Dip. kerrii	F^-	0.84 ± 0.01	0.84 ± 0.02	0.84 ± 0.03	0.78 ± 0.02
	Cl^-	40.65 ± 1.53	32.09 ± 0.88	25.82 ± 0.75	22.47 ± 0.41
	NO_2^-	0.00 ± 0.00	0.45 ± 0.19	0.72 ± 0.20	0.00 ± 0.00
	Br^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	87.37 ± 2.71	68.97 ± 2.25	55.06 ± 1.44	50.13 ± 1.42

Table A-3. Concentrations of different anions in the leachates of various pots at various weeks presented as mean ± standard error (mg L^{-1}).

		Week 4	Week 5	Week 6	Week 7
Ela. tapos	F^-	0.23 ± 0.02	0.26 ± 0.03	0.23 ± 0.03	0.20 ± 0.02
	Cl^-	23.66 ± 4.05	15.07 ± 4.15	21.89 ± 0.53	20.24 ± 2.27
	NO_2^-	1.68 ± 0.20	1.74 ± 0.18	1.45 ± 0.13	0.96 ± 0.10
	Br^-	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	96.42 ± 7.84	76.96 ± 3.67	65.52 ± 3.88	59.05 ± 2.79
Gar. tubifera	F^-	0.42 ± 0.04	0.43 ± 0.02	0.45 ± 0.03	0.41 ± 0.03
	Cl^-	25.96 ± 2.24	27.17 ± 0.96	27.73 ± 1.15	28.27 ± 1.08
	NO_2^-	1.16 ± 0.14	0.84 ± 0.14	1.00 ± 0.18	0.61 ± 0.23
	Br^-	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01
	SO_4^{2-}	33.23 ± 2.75	33.35 ± 2.31	37.82 ± 2.20	33.25 ± 1.68
Hop. ferrea	F^-	0.82 ± 0.02	0.70 ± 0.01	0.68 ± 0.02	0.94 ± 0.10
	Cl^-	33.18 ± 1.69	24.63 ± 1.29	21.20 ± 0.83	28.38 ± 3.12
	NO_2^-	0.31 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.81 ± 0.18
	Br^-	0.15 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	51.18 ± 3.17	74.57 ± 2.84	71.04 ± 2.46	54.02 ± 6.13
Lit. sundaicus	F^-	0.18 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.24 ± 0.02
	Cl^-	44.79 ± 6.22	26.34 ± 5.88	30.53 ± 3.74	27.04 ± 6.03
	NO_2^-	2.04 ± 0.14	1.41 ± 0.33	2.04 ± 0.23	1.46 ± 0.19
	Br^-	0.00 ± 0.00	0.08 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	186.14 ± 16.96	144.78 ± 6.14	138.14 ± 8.73	121.11 ± 7.82
Pae. foetida	F^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.02
	Cl^-	37.00 ± 7.65	30.18 ± 1.59	29.51 ± 3.49	23.84 ± 2.72
	NO_2^-	0.07 ± 0.07	0.69 ± 0.08	0.63 ± 0.23	1.50 ± 0.11
	Br^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	122.81 ± 20.34	89.73 ± 6.14	59.65 ± 7.57	50.74 ± 2.42
Pip. sarmentosum	F^-	0.53 ± 0.02	0.48 ± 0.02	0.48 ± 0.01	0.41 ± 0.00
	Cl^-	20.55 ± 2.39	22.19 ± 0.19	24.11 ± 0.81	22.25 ± 0.42
	NO_2^-	0.83 ± 0.13	0.51 ± 0.15	0.24 ± 0.08	0.00 ± 0.00
	Br^-	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	27.61 ± 0.90	30.27 ± 0.46	29.84 ± 0.75	31.27 ± 0.46

Table A-4. Concentrations of different anions in the leachates of various pots at various weeks presented as mean ± standard error (mg L^{-1}).

		Week 4	Week 5	Week 6	Week 7
Pla. obovata	F^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.01
	Cl^-	58.53 ± 5.87	30.60 ± 2.40	32.02 ± 0.78	22.42 ± 0.91
	NO_2^-	0.10 ± 0.10	0.78 ± 0.10	1.17 ± 0.12	1.17 ± 0.15
	Br^-	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	198.57 ± 15.73	103.95 ± 7.57	82.72 ± 3.68	54.51 ± 3.04
Pre. serratifolia	F^-	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.01
	Cl^-	95.84 ± 7.66	75.18 ± 5.27	80.45 ± 6.55	67.78 ± 3.44
	NO_2^-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Br^-	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.04 ± 0.01
	SO_4^{2-}	164.32 ± 12.04	122.10 ± 10.68	109.82 ± 7.97	89.28 ± 4.43
Sch. elliptica	F^-	0.24 ± 0.01	0.38 ± 0.02	0.31 ± 0.01	0.31 ± 0.02
	Cl^-	60.83 ± 8.35	27.03 ± 3.28	23.77 ± 0.63	0.00 ± 0.00
	NO_2^-	0.21 ± 0.17	0.45 ± 0.18	0.46 ± 0.03	0.00 ± 0.00
	Br^-	0.10 ± 0.08	0.64 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	155.20 ± 24.80	52.53 ± 3.27	41.50 ± 2.88	55.00 ± 4.35
Ste. macrophylla	F^-	0.23 ± 0.01	0.31 ± 0.02	0.27 ± 0.01	0.27 ± 0.02
	Cl^-	97.05 ± 14.63	33.31 ± 2.33	23.24 ± 0.92	20.07 ± 3.40
	NO_2^-	0.07 ± 0.01	0.53 ± 0.18	0.37 ± 0.08	0.28 ± 0.08
	Br^-	0.08 ± 0.01	0.35 ± 0.21	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	118.12 ± 11.36	53.21 ± 2.97	49.78 ± 3.87	114.32 ± 9.61
Syz. leucoxylon	F^-	0.86 ± 0.02	0.75 ± 0.02	0.77 ± 0.03	0.85 ± 0.09
	Cl^-	36.13 ± 1.48	24.79 ± 0.70	18.64 ± 2.41	29.99 ± 3.25
	NO_2^-	0.24 ± 0.18	0.11 ± 0.11	0.00 ± 0.00	0.45 ± 0.22
	Br^-	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
	SO_4^{2-}	70.42 ± 4.89	87.50 ± 5.41	69.78 ± 4.09	75.36 ± 8.73
Syz. myrtifolium	F^-	0.06 ± 0.00	0.06 ± 0.00	0.13 ± 0.05	0.10 ± 0.01
	Cl^-	17.53 ± 2.96	23.53 ± 0.36	19.91 ± 0.36	24.45 ± 0.55
	NO_2^-	0.00 ± 0.00	0.02 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
	Br^-	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
	SO_4^{2-}	37.93 ± 1.49	28.39 ± 1.56	15.98 ± 0.77	89.31 ± 4.65

Table A- 5. Concentrations of different anions in the leachates of various pots at various weeks presented as mean ± standard error (mg L^{-1}).

		Week 4	Week 5	Week 6	Week 7
Tal. tiliaceum	F^-	0.46 ± 0.02	0.33 ± 0.02	0.52 ± 0.03	0.30 ± 0.03
	Cl^-	37.55 ± 6.12	66.73 ± 10.45	30.33 ± 1.58	42.47 ± 4.25
	NO_2^-	1.42 ± 0.43	0.22 ± 0.15	1.80 ± 0.32	0.28 ± 0.21
	Br^-	0.90 ± 0.90	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00
	SO_4^{2-}	26.33 ± 1.44	32.98 ± 2.11	24.89 ± 0.84	35.57 ± 1.42
Tar. odorata	F^-	0.16 ± 0.03	0.17 ± 0.02	0.15 ± 0.02	0.13 ± 0.02
	Cl^-	74.21 ± 10.89	53.66 ± 4.59	47.43 ± 3.17	36.10 ± 1.50
	NO_2^-	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
	Br^-	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
	SO_4^{2-}	108.80 ± 10.42	65.84 ± 4.78	65.84 ± 3.52	54.74 ± 2.42
Tristan. whiteana	F^-	0.25 ± 0.02	0.31 ± 0.03	0.27 ± 0.02	0.31 ± 0.02
	Cl^-	48.38 ± 6.34	28.25 ± 2.22	21.46 ± 1.20	23.61 ± 1.33
	NO_2^-	0.13 ± 0.09	0.04 ± 0.04	0.30 ± 0.11	0.59 ± 0.15
	Br^-	0.05 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
	SO_4^{2-}	73.77 ± 7.50	46.13 ± 4.31	36.63 ± 2.38	49.90 ± 2.50
Tristel. australasiae	F^-	0.53 ± 0.03	0.55 ± 0.02	0.44 ± 0.02	0.50 ± 0.02
	Cl^-	24.39 ± 1.15	24.43 ± 0.66	17.31 ± 0.31	24.55 ± 0.48
	NO_2^-	0.87 ± 0.10	0.74 ± 0.09	1.26 ± 0.09	0.78 ± 0.14
	Br^-	0.05 ± 0.04	0.07 ± 0.05	0.01 ± 0.01	0.00 ± 0.00
	SO_4^{2-}	34.03 ± 1.23	37.73 ± 1.09	24.25 ± 0.81	35.22 ± 0.62