Investigating the exogenous application of 5-Aminlevulinic acid to improve turfgrass (*Lolium perenne* L.) surfaces grown in shade

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ABSTRACT

The quality of turfgrass playing surfaces can be severely compromised when grown in the modern sports stadia environment. Shade from the large grandstands prevent direct sunlight from reaching most of the pitch and Grounds Managers are using lighting rigs to replace natural light. Other solutions are required to reduce the high energy costs of this equipment yet maintain the essential high quality of the playing surfaces. This study investigated the effect of exogenous applications of 100mg L^{-1}e 5-Aminolevulinic acid (5-ALA) to turfgrass *Lolium perenne* L. grown in 100% daylight (Light) and 50% daylight (Shade). Two experiments were setup to investigate a number of parameters. Experient One consisted of turfgrass *L. perenne* grown in tubs containing a sand rootzone overlying gravel to replicate the modern sports pitch construction. Clippings were analysed for chlorophyll content (NDVI), % Dry Weigh and leaf nutrient content (mg kg^{-1}). Experiment Two used the same rootzone and grass seed as in Experiment 1 but grown in 3 inch pots. Fluorescence parameters measurements concentrated on the effects of exogenous applications of 100mg L^{-1} on Photosystem II (PSII): Maximum Quantum Yield (Fv/Fm), Quantum Yield (ØPSII or Fq'/Fm'), and Non-Photochemical Quenching (NPQ). Exogenous applications of 100mg L^{-1} 5-ALA resulted in significant increases in chlorophyll (NDVI) in treated plants compared the Control (non-treated) in both Light and Shade on Days 7 and 14 after treatment, and in Shade on Day 14 after treatment. % Dry Weight increased only on Day 7 after treatment in Treated Shade grown plants. There were significant differences of some nutrients due to 5-ALA treatments: Mg and Zn on Day 0 (4 hours) after treatment; Mn and Zn on Day 7 after treatment. There were some effects on fluorescence parameters, but significant differences were mainly attributed to whether the plants were grown in Light or Shade, not applications of 5-ALA.

Acknowledgements

Thank you to the staff at Sutton Bonington for coping with the relatively unfamiliar subject of turfgrass plants, particularly the laboratory and glasshouse staff for their patience and flexibility in accommodating me. My wife has been the bed-rock of my studies, taking much of the burden of providing an income and allowing me the space to complete my studies.

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CHAPTER One

1.1 Introduction

The main aim of this study is to investigate if exogenous applications of 5-Aminolevulinic acid improve turfgrass surfaces grown in shade, with a focus on Perennial Ryegrass (*Lolium perenne* L.) turfgrass surfaces grown in sports stadia.

My curiosity was sparked in February 2019 when I took the following photograph in my back garden (Fig 1.)

Fig 1. Patch of green grass (outlined in white) taken in February 2019, four months after treatment with a biostimulant in October 2018.

The extra green area in the far corner (marked in white lines) shows where I had treated the grass with a plant extract called 5-Aminolevulinic acid in October 2018 and was showing as extra growth and colour in February 2019. I was intrigued as to what had caused this, e.g. enhanced nutrient uptake, an increase in photosynthesis, or other cause? The area is in a shaded part of the garden and, with my professional interest in supplying football clubs and golf courses with plant biostimulants, I decided to use the subject of growing turf in shade for my MRes project.

1.2 Turfgrass Surfaces grown in Shade

Grass requires light to photosynthesise and to enable turfgrass managers to prepare good quality playing surfaces. Any reduction in light intensity and quality results in reduced photosynthesis in the turfgrass plant and, subsequently, is less tolerant of wear, disease and environmental stress (Turgeon 2008).

Modern sports stadia in the UK are designed for optimum spectator visibility and a safe environment, and more visitor numbers watching sports events in a safe and comfortable environment are key to the commercial sustainability of stadia. This demand has led to the construction of large stadia, but which also results in reduced direct sunlight reaching the pitch (Hunter *et al*., 2009). The playing surfaces in professional sports stadia are of a vastly higher quality than those found 20 and more years ago but the reduction in light from enclosed and roofed stadia causes problems for grass-based playing surfaces, with weakened shoot tissue, reduced growth and sward development, leading to a loss of grass cover and increased surface algal invasion (Dabrowski *et al*., 2015). Tegg and Lane (2004) found that shade stress was the primary outcome under reduced light intensity in stadia, with all turfgrass species used in the study declining in quality as indicated by an increase in thin, succulent vertical growth, and less-dense turf swards.

Figs 2 and 3 were taken by the author and highlight the problems of shade on turfgrass surfaces.

Fig 2. The Etihad Stadium, Manchester City FC, illustrating how the high stadia construction reduces direct light to the playing surface. From the author's private collection.

Fig 3. Sennelager British Army GC, Germany, showing the extent of shade on fairway and green. From the author's private collection.

L. perenne is the most used turfgrass species in UK sports stadia for its ability to quickly germinate from seed, its excellent wear tolerance and quick recovery, and response to fertilisers helping to present a thick sward for play and presentation purposes (Bonos and Huff, 2013; personal correspondence with three Head Grounds Managers: P. Ascroft (Arsenal), T. Stones (Parc de France) and T. Sinclair (Manchester United)). *L. perenne* has a lower tolerance to shade than other turfgrass species, such as *Poa pratensis* spp., *Festuca rubra* spp. and *Agrostis palustris* spp. (Gardener and Goss, 2013), but the importance of *L. perenne* can be seen in its use as the most commonly used grass species in urban areas in Central and Western Europe, with more than 400 turf turfgrass varieties registered in the EU (Dabrowski *et al.,* 2019). Huylenbroeck *et al.* (2009) stress that one of the future challenges for the breeders of turf-grass is the development and use of turfgrass cultivars with superior shade resistance.

The problem caused by shade in modern built sports stadia was highlight in a Special Report by Newell (1995) in which he questions the drive toward building larger stadia without sufficient research on the effects of reduced light conditions on the pitch. He concluded that the quality of pitches would suffer if this progression was continued. Phillips (2005) investigated the effects of stadia on natural grass surfaces and concluded that the only feasible answer was to build stadia with movable pitches. These grass surfaces would be wheeled in and out when required allowing maintenance of the turf to be carried out in full light outside of the stadium. This latter system was tried at the Millennium Stadium in Cardiff (renamed the Principality Stadium) but the loss of car parking space severely curtailed stadium income and it was abandoned. Leaving the pitch in-situ., however, resulted in a serious loss of surface quality as no direct sunlight reached the pitch (Lee Evans, stadium Head Groundsman, personal communication May 2011).

1.3 Current Solutions to Growing Turfgrass Surfaces in Shade

Over the past 15 years the use of high-pressure sodium (HPS) lighting rigs has become extensively used as they provide light to the grass surface inside the stadium (Fig 4). However, the purchasing cost of £90,000 per rig and nine rigs required to cover the pitch (total of £810,000), and £50,000 p.a. per rig to operate over a full-size pitch (total of £450,00 p.a.), even the richest clubs are questioning their continued use (P. Ashcroft (Arsenal Head Groundsman); T. Sinclair (Man. Utd Head Groundsman), personal communication March 2019). These figures also seem to be in contradiction to many club's published energy policies regarding reducing carbon footprint and energy costs, for example: https://www.arsenal.com/news/renewable-energy-partnership-octopus-energy. To mitigate the high costs and energy consumption LED lighting rigs are increasingly being used (Fig 5), which reduce the energy consumption of supplementary lighting. With LED, the wavelength of light can be selected to optimise photosynthesis but some Stadium Managers, however, prefer HPS rigs as they also produce heat which, they say, is beneficial for growing the grass and preparing pitches for play. The cost of providing the heat through under soil heating pipes outweighs any savings from running LED lighting compared to HPS lights (P. Ashcroft, Arsenal FC Head Groundsman, personal communication March 2019).

Fig 4. High Pressure Sodium lighting rigs at The Etihad Stadium, Manchester City FC. (Photo taken on a visit to present to Premier Leagure groundstaff.)

Fig 5. LED lighting showing the red and blue wavelengths. (Photo taken on a visit to present to groundstaff.)

Whilst it can be argued that lighting rigs are essential tools in producing high quality playing surfaces, it is clear that they are not the total answer.

Plant derived biostimulants are increasing being used in the management of high quality turfgrass surfaces. A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms (Jardin, 2015). The global biostimulants market size was valued at USD 2.24 Billion in 2018 and is projected to reach USD 5.69 Billion by the end of 2026, exhibiting a CAGR of 12.4% in the period 2019-2026 (Fortune Business Insights, 2019).

Velez *et al.,* (2014) state that there is growing scientific evidence supporting the use of biostimulants as agricultural inputs on diverse plant species. They point out that cited literature also reveals some commonalities in plant responses to different biostimulants, such as increased root growth, enhanced nutrient uptake, and stress tolerance (Velez *et al.*, 2014).

Increasing research has also identified biostimulants substances that enhance photosynthesis (Yakhin *et a.,l* 2017). Being able to reduce the use of high energy consumption lights with inputs of biostimulants may be an attractive proposition for Turfgrass Managers looking to reduce the costs of creating and maintaining high performance turfgrass surfaces, which also have worldwide public exposure via television.

1.4 Effects of shade on turfgrass plants

The scientific literature shows that shade and low light environments effect turfgrass growth in a number of ways.

i. Light quality

The type of shade has a bearing on turfgrass plant growth. Bell *et al.* (2000) assessed the spectral quality of light in environments caused by deciduous and coniferous trees, from buildings shade and in full sun in a natural environment common to turfgrass growth throughout a growing season. It was found that both deciduous and conifer trees filtered significantly more red and blue quanta than a building, with light filtered by trees containing more light in the red wavelength, and shade from buildings higher in the blue wavelengths. This reduction in the ratio of Red:Far Red wavelengths results in an increase in apical dominance in turfgrass plants with reduced tiller formation and development leading to a loss of density and reduced quality of the playing surface (Dudeck and Peacock, 1992; Fry J and Huang , 2004a; Kebrom and Brutnell 2007).

ii. Physiological effects of shade on turfgrass species

The first physiological symptom of shade in plants is the reduction of the rate of photosynthesis leading to less energy and carbohydrate production. The content of phytohormones ethylene, gibberellin and auxin increase and cause the elongation of stems and petioles and a loosing of the cell wall and elongated cells (Xu, 2011; Tan and Qian, 2003). The results are a more succulent and delicate leaf structure and a thin sward resulting in reduced tolerance to heat, cold, drought, and wear stress, and increased susceptibility to pests and diseases attack (Beard, 1973; Dudeck and Peacock, 1992; Fry and

Huang, 2004; Dabrowski *et al.,* 2015; Fu *et al.*, 2020). The lack of sunlight in shade causes a lack of energy in the plant and is the cause of stress in turfgrass plants through being unable to resist trampling, heat, pests, disease, and is prone to yellowing, and that in low light protective enzymes SOD and POD activity increases and CAT decreases, reflecting the levels of stress the turfgrass is undergoing (Xu, 2011).

The photosynthetic apparatus in shade is more efficient at harvesting light, but it assimilates less CO_2 in comparison with leaves in the sun (Dabrowski *et al*., 2015). Kosugi *et al*. (2010) studied the net ecosystem CO_2 exchange from managed *Poa pratensis* sports fields under various light conditions. This study examined the amplitude of diurnal and seasonal changes in photosynthesis and respiration under various light conditions using an in-situ closed dynamic chamber method. Gross canopy photosynthesis declined under high air, soil, and plant organ temperatures, in accordance with the decline in biomass and ecosystem respiration, and resulted in the decline of the C_3 turfgrass sward in summer. The results showed that respiration of C_3 turfgrass under different light conditions is affected by seasonal plant biomass and not temperature, *and these characteristics of ecosystem respiration are unique to this type of vegetation* (author's emphasis in italics). The interaction between the amount of biomass produced and the balance of photosynthesis and respiration determined the carbon gain and summer decline of turfgrass pitches under different light conditions.

An accumulation of pigments occurs in low light e.g., carotenoids, which are secondary metabolites integrated into light light-harvesting complexes (LHC) along with chlorophyll and have central roles in photo-protection and light harvesting. McElroy *et al.* (2006) studied the response of carotenoids in creeping bentgrass (*Agrostis stolonifera* L.) and found that, in reduced light conditions, zeaxanthin converts to violaxanthin, which acts as a light harvesting antennae pigment. Subjected to high and low irradiance, the results showed that creeping bentgrass accumulates high amounts of β-carotene and lutein under high and low irradiance, which results in higher quality turfgrass surfaces than other turfgrass species when grown in shade.

Huylenbroeck and Bockstaele (2001) carried out a trial on four turfgrass species under shade and found that perennial ryegrass had a faster growth with higher net photosynthesis and quantum efficiency, and a lower dark respiration than red fescue (*Festuca rubra* L.). Total chlorophyll content in *L. perenne* cultivars increased significantly under shade, whereas a reduction in total chlorophyll was observed in *P. pratensis*, and no significant reduction in chlorophyll concentration in all *F. rubra* cultivars. They also observed that the reduction in coverage was due to a decrease in tillering, not to a loss of seedlings at germination.

iii. Morphological effects of shade on turfgrass species

The first morphological signs of turfgrasses grown in shade are leaf elongation and decrease in leaf coverage, with a reduction in tillering of perennial ryegrass (Beard, 1973; Dabrowski *et al.*, 2015). Turfgrass plants in shade show longer stems, high specific leaf areas and longer petioles and grow more vertical, root to shoot ratios decrease, cell walls and cuticles are thinner, and chloroplasts are smaller. These morphological features are developed by the plant to capture more photons in low light environments, with the number and size of chloroplasts decreasing and the number of granum and grana lamella increasing to enhance the cell's ability to capture light (Dudeck and Peacock, 1992)

iv. Nutritional effects of shade on turfgrass species

Nitrogen has a large influence on grasses grown in low light conditions (Baldwin *et al,.* 2009). Between 70 and 80% of nitrogen in leaves exists in the chloroplasts, affecting the assimilation of CO_2 by directly affecting chlorophyll, RuBP/Rubisco, and the structure of photosynthetic organs (Xiong *et al.,* 2017).

The choice of nitrogen fertiliser influences plant growth in shade due to nitrate accounting for a higher energy cost than ammonium nitrogen. The assimilation of 1 mol of ammonium nitrogen (NH_4 ^+N) requires 5 ATP molecules, whereas 1 mol of nitrate nitrogen (NO^3 ^-N) requires at least 15-16 molecules of ATP due to the reduction of nitrate to ammonium in cells by two consecutive processes. First, nitrate is reduced to nitrite by nitrate reductase (NR), then a reduction of the nitrite to ammonium by nitrite reductase (NiR) (Haynes and Goh, 2010). The photosynthetic energy consumed by NO_3 N is 145% more than NH_4^+ N, and plants supplied with NH_4 ^+N in low light conditions have higher chlorophyll and Rubisco content/activity, improved stomatal conductance and higher intercellular CO_2 concentration (Xu, 2011). Nitrate reductase is a light-induced enzyme but in low light is reduced in activity and much nitrate cannot be used by the plant.

Available nitrogen in plants growing in reduced light is used in protein synthesis rather than carbohydrate synthesis, and mowing grass in low light leads to a decline in root numbers due to partitioning of carbon energy from roots to leaves (Tegg and Lane, 2004). Movement of carbohydrates from the root to the leaf provides energy for new tissue growth, but the scarce resources cannot support a high number of tillers. Leaf anatomical structure changes enhance the ability to capture light and improve photochemical reactions in the leaf. Taller, thinner stems and longer internode length are due to an increase in the phyto hormones gibberellin and auxin and the leaves become lighter in colour, are thinner leaf slower in growth, and are aimed at capturing more photons in low light (Xu *et al.*, 2011). Shade plants show higher chlorophyll content, and the ratio of chlorophyll *a* and chlorophyll *b* is lower. The number and volume of chloroplasts reduces but the number of granum and grana lamella are increased, and that tolerance to shade by turfgrass plants is linked to high chlorophyll levels in leaves as a response to low photon flux density (PFD) (Gardener and Goss, 2013)

Table 1 summarizes turfgrass response to shade (adapted from Dudeck and Peacock, 1992).

Table 1. Summary of turfgrass growth response to shade. Adapted from Dudeck and Peacock, (1992)

	Growth response	
Level of expression	**Decreased**	**Increased**
Anatomical	Chloroplasts Cuticle thickness Stomatal density Vascular tissue	Thylakoid and grana stack development
Morphological	Leaf thickness Leaf width Stem diameter Dry weight Horizontal growth habit Stolon number and total length Internode diameters Shoot density Rhizome growth	Root/shoot ratio Leaf area Leaf length Spongy paranchyma tissue Vertical growth habit Plant height Succulence
Nutritional	Growth and yield, but interactions are often observed Carbohydrates	Dark green colour
Physiological	Photsynthesis Respiration rate Compensation point Carbohydrate reserve C/N ratio Transpiration rate Osmotic pressure Flowering	Tissue moisture Lignin content

1.5 5-aminolevulinic Acid (5-ALA)

Photosynthesis has been a target for a long time in crop production research to maximise crop productivity, to alleviate potential starvation in a rising population compounded by rising temperatures, droughts, decreasing soil moisture, and disappointing C_3 crop yield increases from trials with higher concentrations of CO_2 (Paul *et al.*, 2001, Singh *et al.*, 2014). The photosynthetic process, however, is considered to be very susceptible to biotic and abiotic stresses (Feng *et al.,* 2020). The plant extract that is the focus of this study, 5-aminolevulinic Acid (5-ALA), has been reported to both increase photosynthesis and reduce the effects of biotic and abiotic stresses (Akram and Ashraf, 2013; Wu *et al.*, 2019).

5-ALA in plants

5-aminolevulinic acid (5-ALA) is a precursor of all tetrapyrroles in the biological world and, in plants, 5-ALA is in very low concentrations of about 60uM. 5-ALA is the common precursor of chlorophyll, heme, siroheme, vitamin B_{12} and phytochromobilin, has physiological activity as a plant hormone and is known to be effective against the harmful effects caused by various abiotic stresses in plants through over-accumulation (Akram and Ashraf 2013; Wu *et al.,* 2019; Feng *et al.,* 2020).

ALA is synthesised in the stroma of chloroplasts in plants via the Beale, or C_5, pathway (Fig. 6), starting with glutamic acid in the tricarboxylic acid (TCA) cycle, which provides the carbon skeleton (Wu *et al.,* 2019).

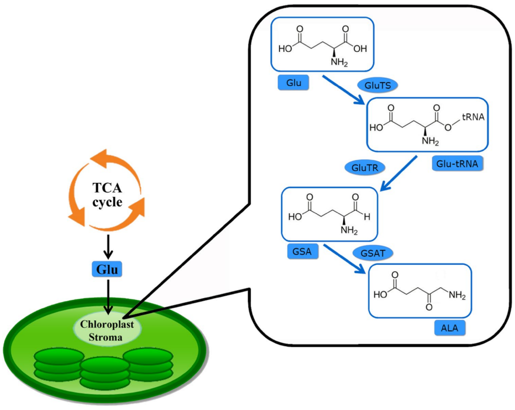


Fig 6. The biosynthesis pathway of ALA in higher plants. The biosynthetic pathway of ALA in higher plants. The main biosynthetic pathway of ALA in higher plants was called Beal pathway or C_5-pathway. This pathway starts from glutamic acid (Glu), which is produced by the tricarboxylic acid cycle (TCA cycle). Glu ligates $tRNA^{Glu}$ and generates Glu– tRNA are catalyzed by GluTS. GluTR then acts a catalyer that converts Glu–tRNA into GSA. At last, catalyzed by GSAT, ALA is created in stroma of the chloroplast. (Wu *et al.,* 2019)

Chlorophyll is created when the enzyme Mg-chelatase activates, starting at Proto IX in the metabolic pathway (Fig.7). Protochlorophyllide is deoxidized to form Pchlide, and Chlorophyllide is formed. Chlorophyll *a* and chlorophyll *b* are formed under the action of the enzyme chlorophyll synthase.

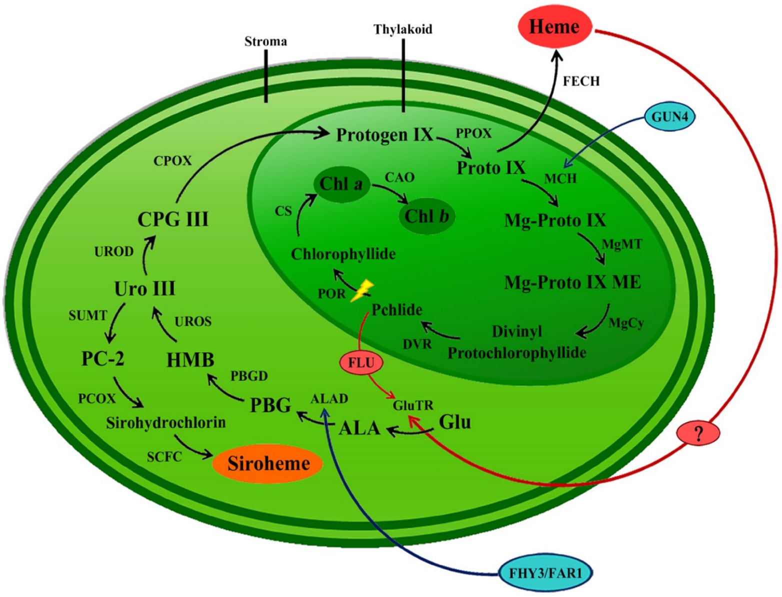


Fig 7. The downstream metabolism of ALA and regulatory factors among metabolic pathways. ALA is the common precursor of chlorophyll, heme and siroheme. Feedback inhibition effect plays an important regulative role in the pathway, where the pathway is associated with the positive regulators GUN4 and FHY3/FAR1 proteins, and negative regulators FLU protein. Chlorophyll synthesis starts at Mg-Proto IX. (Wu *et al.,* 2019)

5-ALA as a plant treatment

Exogenous applications of ALA are made with 5-amino-4-oxypentanoic acid hydrochloride (also known as 5-aminolevulinic acid or 5-ALA), a linear five-carbon compound with the structural formula $C_5H_9NO_3$-HCL (Fig 8). 5-ALA is a white to off-white, odourless crystalline solid, has an atomic weight of 176.59, and is very soluble in water (Akram and Ashraf 2013).

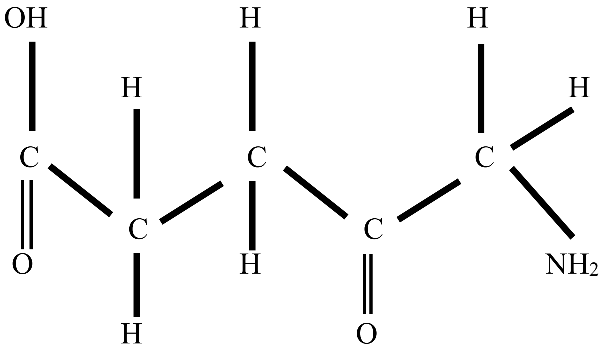


Fig 8. Structural formation of 5-aminolevulinic acid. *From*: Akram and Ashraf (2013)

5-ALA induces tolerance to various abiotic stress conditions and exogenous applications have been shown to help plants mitigate the effects of various abiotic stresses such as herbicide damage, shade, cold, drought, salt, heavy metals and water logging. In low concentrations (0.06-0.6mmol/L) 5-ALA is a non-toxic plant growth promoting hormone that regulates the growth and development of higher plants by enhancing their photosynthetic activities (Hara *et al.* 2011). High concentrations (5-40 mmol/L) of 5-ALA, however, promote the accumulation of Reactive Oxygen Species (ROS), i.e. superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicle ($^{\bullet}OH$), and can be used as a biodegradable herbicide (Hara *et al.,* 2011; Korkmaz, 2012). Endogenous ALA can be increased with exogenous applications of 5-ALA, and the primary fluorescence of chlorophyll and the electron transfer rate of light harvesting pigments are enhanced resulting in increased activity of ribulose-1,5-biphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase), which promote photosynthesis (Wang *et al*., 2018; Wu *et al.,* 2019). Exogenous applications of 5-ALA increase the accumulation of free sugars, i.e. glucose, fructose and sucrose, by mobilizing starch-degrading enzymes and Hara *et al.* (2011) state that this is the main mechanism by which 5-ALA promotes the growth of plants. Xu *et al.* (2011) concluded that low volume exogenous applications of 5-ALA at 10 and 100 mgl^{-1} promoted chlorophyll, soluble sugar content, and the activities of three flavonoid and anthocyanin content in the leaves of *Ginko biloba*. Exogenous treatment of 5-ALA also increases heme content in plants and Wu *et al.* (2019) hypothesized that this enhances oxidative stress resistance and tolerance in plants and increases photosynthesis which produces more carbohydrate in the plant, leading to more energy to react against abiotic stresses. These stresses can cause damage to the configuration of chloroplastids and chloroplasts, swollen grana, and deformed thylakoids but have been reversed by applications of 5-ALA through regulation of photosynthesis, increased nutrient uptake, antioxidant defence systems and osmoregulation (Akram and Ashraf 2013; Wu *et al.,* 2019). 5-ALA could be used as a biofertilizer due to its promotive effects on growth and yield of several crops under various abiotic stresses (Korkmaz, 2012; Phour *et al.,* 2018).

When under biotic and abiotic stresses Reactive Oxygen Species (ROS) in plants increase and interfere with normal metabolic processes. Treatments with 5-ALA suppresses excess hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) and increases antioxidant scavenging enzymes such as superoxide dismutase, catalase, ascorbate and peroxidase by enhancing antioxidant defence systems. 5-ALA increased photosynthesis capacity, regulating antioxidant enzyme gene expression and proline accumulation (Zhang and Wang, 2015). Al-Ghamdi and Elansary (2018) combined weekly treatments of seaweed extracts (SWE) at 7 mlL^{-1} and/or 5-ALA at 3, 5 and 10 ppm on *Asparagus aethiopicus*. The joint application of SWE and 5-ALA produced the best morphological results when under stress conditions, with a synergistic effect between these two treatments. Significant increases were found in the CAT and SOD antioxidant enzymes, and also in the accumulation of phenol secondary metabolites and soluble carbohydrates. Total increases in chlorophyll, the rate of photosynthesis and proline content were found in SWE + ALA treatments.

Zhang *et al.* (2013) found that application of 5-ALA improved the following chlorophyll fluorescence parameters: photochemical efficiency (Fv'/Fm'), PSII actual photochemical efficiency (ØPSII), and photochemical quench coefficient (qP), but the non-photochemical quenching coefficient (NPQ) was decreased, indicating that PSII photochemical activity can be repaired after being subjected to salt stress. Saline water is an increasing problem in growing crops in many parts of the world due to shortages of potable water (Al-Ghamdi and Elansary, 2018).

5-ALA combined with nutrient fertilisers could be responsible for increases in mineral nutrient content in plants. Xu *et al.* (2010) reported increases in plant biomass, chlorophyll content, photosynthetic rate, stomatal conductance, increases of N, P, K and Ca concentrations, soluble starches, sugars, vitamin C, flavonoids and puerarin. Hotta *et al.* (1997) found that 5-ALA stimulated an accumulation of chlorophyll, but the promotion of photosynthesis was primarily caused by 5- ALA treatments stimulating the activity of nitrate reductase in plant tissue that promoted growth. Korkmaz (2012) reported Iwai *et al.* (2003) who found exogenously applied 5-ALA in combination with high nitrogen fertiliser increased up to 9% more yield than control plants by causing plants to utilise 16% more NO_3 from the nutrient solution. Wu *et al.*

(2019) hypothesized that an increase in the biosynthesis of siroheme through applications of 5-ALA is linked to an increase in the uptake of nitrogen and sulphur. Siroheme plays a crucial role in reduction actions of these nutrients in plants.

Effects of 5-ALA on plants grown in shade/low light

Exogenous treatments of 5-ALA to plants grown in low light and shade has been shown to increase photosynthetic gas exchange and photochemical efficiency (Sun *et al.,* 2009), and also increase antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) particularly of those present near the reaction centres of PSI (Akram and Ashraf,2013). This leads to an increase in the electron transport rate and alleviates photosynthetic inhibition under low-light conditions. Higher levels of chlorophyll were attributed by Guo *et al.* (2012) to applications of 30mg L^{-1} 5-ALA to tomato seedlings alleviating the down-regulation of electron transferring of PSII reactive centres that were being inhibited by sub-optimal light.

Effects of 5-ALA on turfgrass species

There are only a few papers written on the use of 5-ALA on turfgrass species. Hotta *et al.* (2000) investigated the effects of 5-aminolevulinic acid (ALA) on the growth of Manilla Grass (*Zoysia matrella*) and Bentgrass (*Agrostis* spp.) in favourable environmental conditions. Foliar application of 30-100ppm 5-ALA prevented discolouring of Manila Grass in early winter and promoted spring growth in the following spring (similar to the author's initial prompt, Fig 1.). The effectiveness of ALA was also observed in bentgrass, when the compound was applied at 5 -10ppm. The authors concluded that the effectiveness of ALA on these turfgrasses might be caused by the properties of stimulation of photosynthesis and suppression of photorespiration.

Turfgrass plants treated with 5-ALA have shown less physiological damage in salt affected soils by the suppressing of Na^+ accumulation and enhanced photosynthesis, respiration, osmotic regulation and antioxidant defences. Yang *et al.* (2014) treated creeping bentgrass (*Agrostis stolonifera*) with weekly foliar applications of 0.5 mg L^{-1} concentration 5-ALA and were irrigated with 200mM NaCl for 28 days. Control and 5-ALA treated plants decreased in quality, but the 5-ALA plots showed less deterioration with less cell membrane damage, accumulation of organic acids, amino acids and sugars. 5-ALA treatments mitigated physiological damage by suppressing Na^+ accumulation and enhanced physiological and metabolic activities related to photosynthesis, respiration, osmotic regulation and antioxidant defense. There were also increase sin accumulations of organic acids, amino acids and sugars. Exogenous applications of 5-ALA were found to induce the biosynthesis of endogenous 5-ALA, and also had a direct effect on the health of PSII proteins when under osmotic stress with higher values of qP. Another significant effect caused by exogenous 5-ALA applications was the higher rate of Rubisco in osmotic-stressed in pre-treated plants. The authors suggested that 5-ALA could increase the phases of carbon fixation and RuBP regeneration in the Calvin-Benson Cycle and concluded that 5-ALA pre-treatment under osmotic stress conditions primarily affected the transcripts associated with photosynthesis, carbon fixation in photosynthetic organs, porphyrin and chlorophyll metabolism.

Drought stress is another important turfgrass plant stress that 5-ALA has been found to alleviate. Nui *et al.* (2017) studied the effects of 5-ALA treatments on antioxidant metabolism and gene expression in *P. pratensis* seedlings induced with drought stress. Free radicals such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) significantly increase under drought, but Niu *et al.* (2017) found that treatments on *P. pratensis* with 10 mg L^{-1} 5-ALA showed reduced oxidative damage through enhanced activity of the anti-oxidants SOD, CAT and APX. A pre-treatment of seed before planting increased turf quality and leaf relative water content and reduced the production of reactive oxygen species during the 25-day period of drought stress. The pre-treatments weakened stress-induced photoinhibition and increased photosynthesis, carbon fixation was significantly enriched which maintained metabolic energy. Drought stress induces an imbalance between the light reactions and the Calvin-Benson cycle, which results in the production of ROS by the transfer of electrons to molecular oxygen. In a follow up study, Nui and Ma (2018) used RNA sequencing in *P. pratensis* to explore the molecular roles played by 5-ALA during osmotic stress. They found that photosynthesis, carbon fixation and chlorophyll biosynthesis were enriched by the induction of a series of enzymatic reactions. The fluorescence parameters of ØPSII, ETR and qP showed increases in drought stressed and treated samples compared to untreated, with NPQ values significantly less in treated compared to untreated samples. The authors concluded that the results suggest that a combination of the regulation of the transcriptome and the physiology of chlorophyll biosynthesis, photosynthesis and the Calvin Cycle were involved in the protective mechanism of 5-ALA pre-treatment in response to osmotic stress. The application of 5-ALA might improve drought tolerance and turfgrass surface quality through reducing oxidative damage and increasing non-enzyme antioxidant levels, and by increasing antioxidant enzyme levels at transcriptional and post-transcriptional levels.

Heat stress of turfgrass surfaces is a problem in many parts of the world, especially if C_3 turfgrass species are used in above optimal conditions, e.g. the 2022 World Cup to be played in Qatar. Katuwal *et al.* (2020) applied 100 mg L^{-1} 5-ALA to Tall Fescue (*Festuca arundinacea* Scheb.) to study the effects during heat stress. Compared to untreated, plants treated with 5-ALA showed the best response with higher chlorophyll levels, total chlorophyll contents, turf quality and % green cover, and lower electrolyte leakage. Chlorophyll levels and photosynthetic rates were higher in treated plants compared to the control.

1.6 Conclusion of review

Perennial Ryegrass (*L. perenne* L.) is extensively used in sports stadia due to: its ability to germinate quickly from seed and establish a playing surface in a few weeks; high tolerance and quick recover from wear; high shoot density; response to fertiliser in creating and maintaining an aesthetically looking sward for television (Thorogood, 2003; personal communications with Stadia Managers). The low light in stadia, however, has a deleterious effect on the quality of the playing surface due to *L. perenne* having a low tolerance to shaded environments and pitch managers need to use expensive lighting rigs to establish and maintain the playing surface. Treatments of turfgrass species with 5-ALA have been used to mitigate heat and salinity stresses, which have shown increases in photosynthesis and chlorophyll content in treated turfgrass plants. To date, however, no study has been carried out on the effects of exogenous treatments of 5-ALA on perennial ryegrass in shade conditions.

Two experiments were conducted to measure various aspects of the effects of foliar applied 5-ALA on *L. perenne*. Akram and Ashraf (2013) concluded that soil applied 5-ALA is not economically feasible as relatively large amounts are required to make significant differences. The concentration of 100mg L^{-1} of 5-ALA was chosen as it is the maximum amount already tried on turfgrass plants (Liu, 2016; Anjum *et al*, 2016)

The objective was to determine if foliar applications of 100mg L^{-1} of 5-Aminolevulinic acid can improve the quality of *L. perenne* pitches in a shaded environment.

Chapter TWO

Materials and Methods

Two experiments were conducted to investigate the effects of exogenous applications of 100mg L^{-1} 5-Aminolevulinic acid on turfgrass *L. perenne* sp.

2.1 Experiment One

Experiment 1 was set up in a glasshouse at the University of Nottingham, Sutton Bonington campus, map coordinates 52.8313° N, 1.2512° W, as a replicated trial of turf type perennial ryegrass grown in tubs to replicate modern football stadia rootzone construction and fertiliser inputs, treated and non-treated with 100 mg L^{-1} 5-Aminolevulinic acid in 100% daylight (Light) and 50% full daylight (shade).

2.1.1 Materials

Plastic tubs with a volume of 42L, purchased from https://www.manutan.co.uk/en/key/strata-storage-boxes-42l-pack-of-10 were selected (Figs. 9), holes drilled in the bottom for drainage, 50mm of 6mm grit placed in the bottom followed by 30cm of consolidated rootzone, purchased from Mansfield Sands, Sandmartin House Oak Tree Lane, Mansfield NG18 4LF. Limitations on the quantitiy of available rootzone material meant that twelve tubs were prepared, i.e. 3 replicates per treatment.

Fig 9. L-R: The plastic container selected for the trial, holes drilled in the base, layer of 6mm grit consolidated rootzone

The particle sizes and depths of the grit and rootzone are critical in the construction of golf and sports turf surfaces. The United States Golf Association (USGA) Recommendations For A Method of Putting Green Construction (https://archive.lib.msu.edu/tic/usgamisc/monos/2018recommendationsmethodputtinggreen.pdf) is the turfgrass industry accepted criteria for golf greens and sports pitches constructed a to create a perched water table. Bridging of the sand rootzone over the grit is only possible when the particle sizes of both materials are in the correct ratio and percentage quantity, which prevents the need for a blinding layer that adds to costs of construction. The bridging between the layers sand and gravel causes water to stop at the interface and build up a saturated layer called a perched water table. When the weight of water is great enough, designed to be 200-250mm deep, the water will then move into the gravel layer and drain away, allowing air entry at the surface.

The rootzone was supplied by Mansfield Sands Ltd and follows the USGA recommendations for the range of sand particle sizes (https://archive.lib.msu.edu/tic/usgamisc/monos/2018recommendationsmethodputtinggreen.pdf):

1. *Drainage layer gravel:*

Bridging Factor	$\frac{\text{D15 (gravel)}}{\text{D85 (rootzone)}}$	≤ 8
Permeability Factor	$\frac{\text{D15 (gravel)}}{\text{D15 (rootzone)}}$	≥ 5
Uniformity Factors	$\frac{\text{D90 (gravel)}}{\text{D14 (gravel)}}$	≤ 3

100% passing a 12-mm screen ≤10% passing a 2-mm screen ≤5% passing a 1-mm screen

2. Recommended Particle Size Distribution for a Putting Greens Rootzone Mixture (https://archive.lib.msu.edu/tic/usgamisc/monos/2018recommendationsmethodputtinggreen.pdf)

PARTICLE	**DIAMETER**	**SIEVE**	**% BY WEIGHT**
Coarse gravel	>4mm	No. 5	0%
Fine gravel Very coarse sand	2.0 – 3.4 mm 1 – 2 mm	No. 10 No. 18	≤ 3% gravel ≤ 10% combined in this range
Coarse sand Medium sand	0.5–1.0mm 0.25 – 0.5 mm	No. 35 No. 60	≥ 60% of the particles in this range
Fine sand	0.15 – 0.25 mm	No. 100	≤ 20%
Very fine sand	0.05 – 0.15 mm	No. 270	≤5%
Silt	0.002 – 0.05 mm		≤5%
Clay	< 0.002 mm		≤3%
Total fines	Very fine sand + silt + clay		≤ 10% combined
Coefficient of Uniformity (D60/D10)		1.8 - 3.5 2.0 - 3.5 2.0 - 3.5	Rootzone mixtures with peat Rootzone mixtures with inorganic amendments Pure sand rootzone mixtures

Fig. 10 shows a profile of the USGA Recommendations for a Method of Putting Green Construction.

Fig 10. USGA rootzone overlying gravel showing turfgrass roots (*Lolium perenne* spp. L.) and demonstrating a perched water table, with saturated zone near the gravel and drier rootzone (air entry) near the surface. Taken on 28th Oct 2019 at The University of Nottingham - Sutton Bonington Campus, Sutton Bonington, England. From the author's private collection.)

Twelve tubs containing the gravel and rootzone were placed in a glasshouse in two rows of six (Fig 11). Each tub was sown with a blend of turfgrass cultivars, of perennial ryegrass purchased from Rigby Taylor Ltd, with the following cultivars (Fig 16);

25% Eurocordus	30% Europitch
25% Eurosport	20% Columbine

This blend is supplied to a number of English Premier League football clubs by Rigby Taylor Ltd.

Fig 11. L-R: Containers filled with rootzone, list of cultivars of *L.perenne* L. in the blend, seeded container.

A pre-seed fertiliser, purchased from Rigby Taylor Ltd, NPK 8:12:8 was spread on each tub and lightly raked into the surface 20mm. The seed was sown at a rate of 5.852g per tub, the equivalent of 350 kg/ha and consolidated (Fig 10).

Natural daylight was supplemented with over head lighting from high-pressure sodium lamps, suspended two meters above the tubs and timed to provide 16 hours of daylength and eight hours of darkness including natural dayligth. The seed germinated after two days (Fig12).

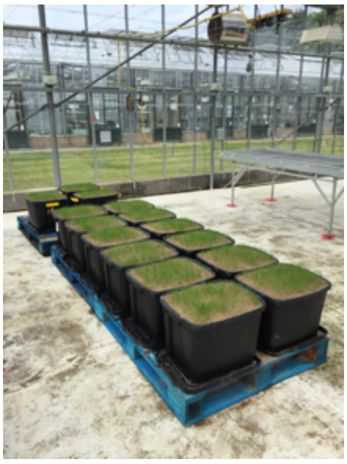


Fig 12. Grass seed germination

2.1.2 *Maintenance*

Mowing was carried out with an electric hedge cutter at a height of 30mm using a frame as a guide (Fig 13). The frame rested on top of the tubs with the top of the frame 30mm above the top of the tubs. When cutting, the grass the cutter was placed on top of the guide rails, giving a 30mm height of cut.

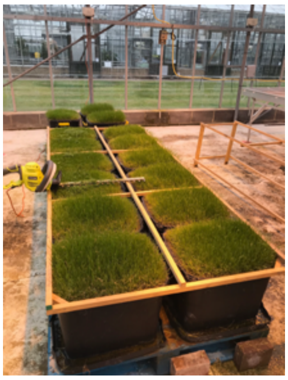


Fig 13. Cutting the grass with an electric hedge trimmer and guide frame

Fertilising was a combination of granular and liquid fertiliser and replicated current practices at top level sports stadia, e.g. Premiership football clubs (personal communications with three Premiership club Head Grounds Managers). The granular fertiliser was an organic based NPK 15:2:12, applied at the equivalenet of 350 kg/ha (5.016 g per tub) every two weeks from the 9th July to 17th September 2019. Nutrients in kg/ha equivelant are calculated to be N- 330, P- 44, K- 264.

A liquid fertiliser, called LGL Pro from Liquid Gold Leaf Ltd, contained N,P,K, Mg, Ca, B, Cu, Fe, S, Mn, Mo, Zn, with all minerals being fully soluble, plus seaweed extract and cross lamina technology. LGL Pro was diluted to 5% and applied at equivelant of 2ml m^{-1} every two weeks from 11th June to 17th September 2019. Nutrients applied from Liquid Gold Leaf in kg/ha equivelant are calculated to be N- 19, P- 11, K- 34.

Total NPK nutrients applied to the tubs including the pre-seed granule (kg/ha equivelant): N- 377, P- 97, K- 326, Mg- 3, Ca- 3.

Water was supplied by an over head irrigation sprinkler set at 30mm per seven days, split into four evenly timed cycles per 24 hours via a Hunter irrigation controller. The water was distilled to remove all other nutrients in the mains water.

The grass was deemed to be ready to apply treatments eight weeks after sowing (Fig 14).

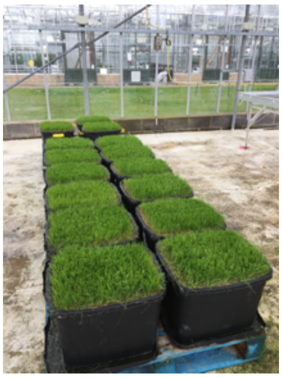


Fig 14. Grass growth after eight weeks.

2.1.3 *Experimental design*

The steel framework of the glasshouse and the overhead lighting and irrigation equipment cast shadow from the sun over the experiment at various times of the day. To minimise the effects of this varying shade the experiment was designed in a split block arrangement with one row of six kept in light and the other row covered with green hessian fabric (Fig 15).

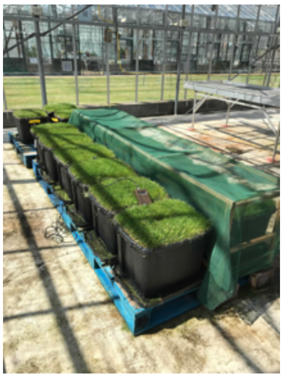


Fig 15. Tubs in shade and full light

Three tubs from each of the shaded and full light rows were randomly selected for treatment (Fig 16).

C	T	C	T	T	C
T	C	C	T	C	T

Fig 16. Layout of tubs: Shade (grey) and full light (white) tubs treated (T) and non-treated (C)

Measurements of light and shade were taken, with 59.8 mol/m^2 s^{-1} in shade and 123 mol/m^{2}s $^{-1}$ in full sunlight, at midday 8th July 2019 (Fig. 17).

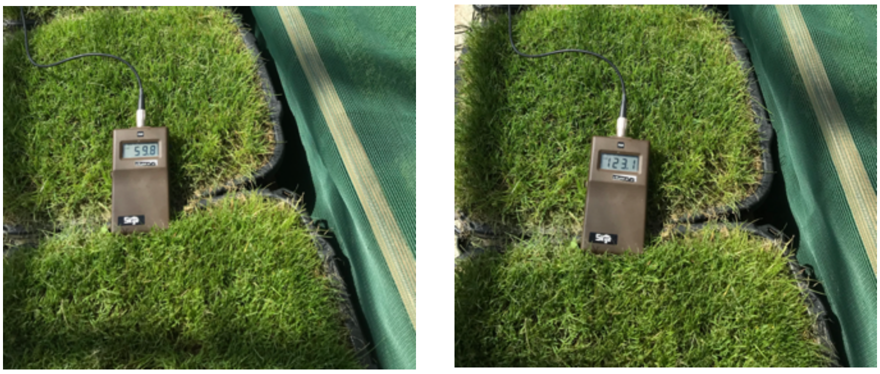


Fig 17. PAR reading in shade - 59.8 m/m^{2}s^{-1}, in full light – 123.1 m/m^{2}s^{-1}

There is little published data on dilution rates for 5-ALA treatments on turfgrass surfaces, but an overview of 5-ALA treatments on various plants in Yue We *et al.* (2019), treatments on turf type creeping bentgrass (*Agrostis stolonifera* L.) by Yang *et al*. (2014), and on watermelon seedlings grown in shade (Sun *et al*., 2009), showed effective treatments at 100-300 mg L^{-1}. A dilution rate of 100 mg L^{-1} was chosen to investigate the effects of 5-ALA treatments. Eight weeks after sowing seed a solution of 100mg L^{-1} of 5-ALA was prepared and applied to the treated tubs on the 23rd July 2019 using a hand- pump pressurised sprayer. Time to spray 100ml was taken and the solution was calculated to apply the equivalent of 400 L/Ha of solution by walking at a timed rate over the distance of the prepared tubs (a standard rate for applying turfgrass treatments), i.e. 40 ml/m^2 = 6.7ml per tub.

2.1.4 Measurements for Experiment One

Samples were taken 0, 7 and 14 days after treatment and subjected to the following:

- % Dry Weight
- Normalized Difference Vegetation Index (NDVI) as measured using the Field Scout CM1000, manufactured by Spectrum Technologies Ltd, Bridgend.
- Nutrient content (mg kg^{-1}) in leaf clippings collected after mowing at 30mm height of cut and measured using an ICP-MS at University of Nottingham, Sutton Bonington..

i. % Dry Weights

After cutting the samples at 30mm height of cut it was not possible to collect all the grass clippings due to some clippings falling onto the floor or to the base of the sward and could not be collected for weighing. This means that direct comparisons of clippings fresh and dry weights between plots could not accurately be made so it was decided to compare the % of dry weight from collected samples of each plot. This method agrees with Hunter *et al.* (2009).

Samples of clippings were collected on day 0 (four hours after treatment), day 7 and day 14 after treatment with 5-ALA, and fresh weight recorded. After drying for 72 hours at 40^0C the clippings were weighted and % Dry Weight (g) calculated using the equation:

$$\frac{\text{Dry weight (g)}}{\text{Fresh weight (g)}} \text{ x } 100$$

The means were subject to 2-Factor Analysis of Variance;

i. Full daylight v 50% daylight (shade)

ii. 5-ALA treated v 5-ALA non-treated

followed by Duncan's Multiple Range Test ($P \leq 0.05$), carried out with statistical software: VSN International (2020). Genstat *for Windows* 21st Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk. The Null Hypothesis was that exogenous applications of 5-ALA do not increase % Dry Weights compared to non-treated samples. The alternative Hypothesis is that exogenous applications of 5-ALA increase % Dry Weight content.

ii. Normailsed Difference Vegetation Index

Fig 18 shows a portable spectroradiometer (Field Scout CM1000, Spectrum Technologies Ltd, Bridgend) being used to give a value for chlorophyl index. When the trigger of the device is pressed the target area is defined, and the Chlorophyll Meter senses light at wavelengths of 700 nm and 840 nm to estimate the quantity of chlorophyll in leaves. The ambient and reflected light at each wavelength is measured. Chlorophyll *a* absorbs 700 nm light and, as a result, the reflection of that wavelength from the leaf is reduced compared to the reflected 840 nm light. Light having a wavelength of 840 nm is unaffected by leaf chlorophyll content and serves as an indication of how much light is reflected due to leaf physical characteristics such as the presence of a waxy or hairy leaf surface. A chlorophyll index value (0 - 999) is calculated from the measured ambient and reflected light data. (Facundo Carmona, Raúl Rivas and Diana C. Fonnegra, (2015). The NDVI is calculated by the succeeding equation: (NIR–R)/ (NIR+R), where R is the reflectance in the red band and NIR is the reflectance in the near-infrared band.

Fig 18. Using the Field Scout CM1000 Chlorophyll Meter. (From author's library)

Nine readings, from a distance of 50–60 cm above and vertical to the canopy, were taken from each tub and means calculated.

The means (g) were subject to 2-Factor Analysis of Variance;

iii. Full daylight v 50% daylight (shade)

iv. 5-ALA treated v 5-ALA non-treated

followed by Duncan's Multiple Range Test (P ≤ 0.05), carried out with statistical software: VSN International (2020). Genstat *for Windows* 21st Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk.

The Null Hypothesis was that exogenous applications of 5-ALA do not increase chlorophyl index values. The alternative Hypothesis is that exogenous applications of 5-ALA increase leaf nutrient content.

iii. Leaf nutrients

Samples from clippings from each tub were taken on Days 0, 7 and 14 after treatment, and prepared for analysis in the Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Thermo Fisher Scientific iCAPQ, Thermo Fisher Scientific, Bremen, Germany) with the following procedure:

a. Subsamples of 0.2g (0.1995 – 0.2050g tolerance) of leaf were digested using a microwave system comprising a Multiwave Pro with a 41-vessel 41HVT rotor (Anton Paar).
b. Leaf material was digested in 6 mL 70 % Trace Analysis Grade HNO3.
c. Microwave settings as follows: power = 1500 W, temp = 175 °C ramp, 175C hold, 55C cooling
d. Two operational blanks were included in each digestion run. Duplicate samples of certified reference material (CRM) of leaf (Tomato SRM 1573a, NIST, Gaithersburg, MD, USA) were included ; laboratory reference material (LRM) from pooled / freeze-dried Brassica napus leaves was also used for later digests.
e. Following digestion, each tube was made up to a final volume of 24 mL by adding 18 mL Milli-Q water and transferred to a 25 mL universal tube (Sarstedt Ltd., Nümbrecht, Germany) and stored at room temperature.
f. Leaf digestates were diluted 1-in-10 using Milli-Q water prior to elemental analysis. The concentrations of 28 elements were obtained using inductively coupled plasma mass spectrometry (ICP-MS; Thermo Fisher Scientific iCAPQ, Thermo Fisher Scientific, Bremen, Germany); Ag, Al, As, B, Ba, Ca, Cd, Cr, Co, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Ti, U, V, Zn.

The means for leaf nutrient content (mg kg^{-1}) were subject to 2-Factor Analysis of Variance;

i. Full daylight v 50% daylight (shade)

ii. 5-ALA treated v 5-ALA non-treated

followed by Duncan’s Multiple Range Test ($P \leq 0.05$), carried out with statistical software: VSN International (2020). Genstat *for Windows* 21st Edition, VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk. The Null Hypothesis was that exogenous applications of 5-ALA do not increase leaf nutrient content. The alternative Hypothesis is that exogenous applications of 5-ALA increase leaf nutrient content.

2.2 Experiment Two

Experiment 2 used the same grass species and cultivars as in Experiment 1 but grown in 3 inch pots with four replications of 100% daylight (Light) and 50% full daylight (shade), treated and non-treated with 100 mg L^{-1} 5-Aminolevulinic acid.

2.2.1 Materials

On the 18th July 2019, twelve 3-inch plastic plant pots were filled with the same rootzone as in Experiment One, seeded with the same grass seed, fertilised and watered for four weeks (Fig 19). The grass was cut at 40mm once per week with handheld scissors.

Fig. 19. Pots after four weeks of growth and 10 days after treatment. Plants grown in Shade (50% daylight) are larger with greener leaves than those grown in Light (100% daylight)

After four weeks growth a solution of 100mg L^{-1} 5-aminolevulinic acid was prepared and applied to shade and light pots tubs, to the following arrangement (Fig. 20).

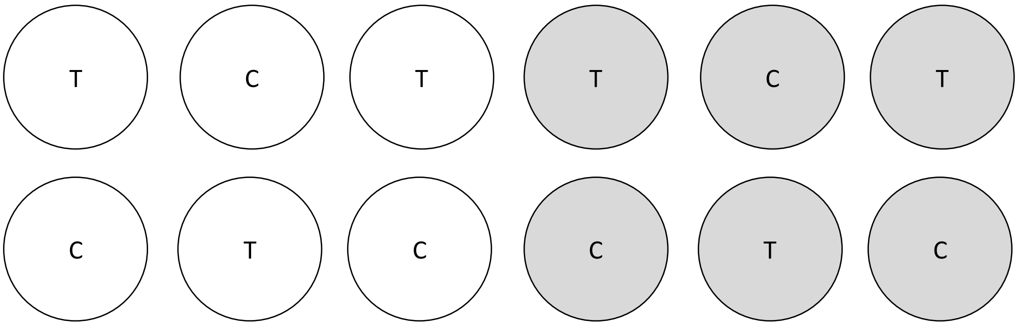


Fig. 20 Arrangement of Experiment Two pots. Grey shaded pots are in 50% full daylight (shade), white pots are grown in full light. T = treated with 5-ALA C = non-treated pots

2.2.2 Measurements of Experiment 2

Samples were taken 0, 2, 4, 8 and 10 days after treatment and subjected to the following:

- A series of readings taken by a fluorescence imager (Light isolated and gas proof Open Fluorcam from Photon Systems Instruments, Czech Republic) on fluorescence and heat emitted by the plants under treatment:
    - PSII Operating Efficiency ØPSII (or *Fq'/Fm'*)
    - Maximum Quantum Efficiency of PSII photochemistry (Fv/Fm)
    - qL (Fraction of open PSII reaction centers)
    - Non-Photochemical Quenching (NPQ)

Experiment Two was conducted in small pots to enable them to be placed in the Fluorescence Imager.

i. Air temperate and Solar Irradiance

Air Temperature and Solar Irradiation levels were taken from the weather station records at the University of Nottingham (Sutton Bonington) for the period of the experiment. The significance of these results are discussed at the end of Chlorophyll Fluorescence results section.

ii. Chlorophyll fluorescence

Light absorption is carried out by light-harvesting pigment-protein complexes (LHCs) and results in singlet-state excitation of a Cl *a* molecule. This excitation state returns to ground state via one of several pathways (Müller et. al, 2001) and Fig 21:

i. Re-emitted as Cl fluorescence (Chl-F)
ii. Transferred to reaction centers for driving photosynthesis
iii. De-excited by thermal dissipation processes (NPQ)
iv. Decay via the triplet state (^{3}Chl*), which can transfer energy to ground-state O_2 that generates singlet oxygen ($^1O_2$), a highly damaging Reactive Oxygen Species.

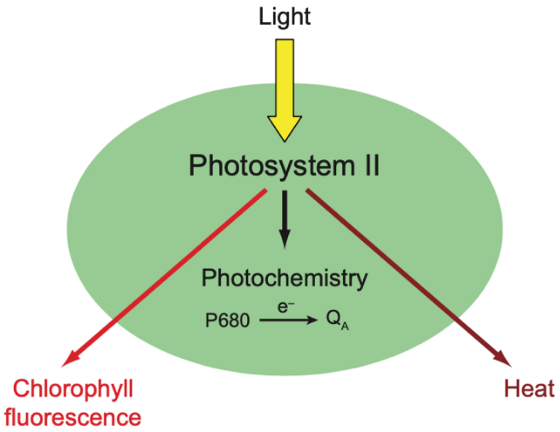


Fig 21. From Baker (2008), illustrating the possible fate of light energy absorbed by photosystem II (PSII). Absorbed light energy can be used to drive photochemistry by transferring an electron (e^-) from the reaction centre chlorophyll (P680) to the primary acceptor, Q_A. Light energy can also be lost from PSII as heat (NPQ) or chlorophyll fluorescence. All three processes are in competition for excitation energy and if the rate one process increases the rates of the other two will decrease.

Light is absorbed by PSII and the electron is accepted by a carrier, or plastoquinone, called Q_A. Once Q_A has accepted an electron it is not able to accept another and the reaction centre is said to be 'closed' until it has been passed onto another electron carrier called Q_B. The portion of reaction centres at any point in time leads to an overall reduction in photochemistry efficiency and to a subsequent increase in fluorescence, which can be measured (Maxwell and Johnson 2000).

Measuring photosystem II (PSII) chlorophyll fluorescence is non-invasive, giving low cost information about the efficiency of photochemistry and heat dissipation. It is a relatively easy technique to gain information on the effects of biotic and abiotic stress on photosystem II (PSII) under different light and other environmental conditions and shows a high correlation with photosynthetic rates, being sensitive to minor alterations in plant metabolism and provides information on the interactions of plant-stress factors. ØPSII provides a rapid method of measuring the PSII operating efficiency, which can be used to measure linear electron flux through PSII and as an indicator of i the quantum yield of CO_2 assimilation by the leaf. (Maxwell and Johnson, 2000; Baker, 2008; Murchie and Lawson, 2013; Pérez-Bueno, Pineda and Barón, 2019). The interpretation of the measurements, however, is more difficult. Fluorescence values on their own have little or no meaning and a well-defined reference state for plants being examined is needed to allow an appropriate interpretation of the data (Kalaji *et al.,* 2014).

2.2.3 Chlorophyll fluorescence of turfgrass species

There are few examples of published research on using chlorophyll *a* fluorescence to measure effects of treatments and/or stress on turfgrass varieties of grass. A relevant piece of work was carried out by Dabrowski *et al.* (2017) measured delayed fluorescence and 820 mn light reflection measurements on salt treated *L. perenne*. Dabrowski *et al,* (2019), measured chlorophyll *a* fluorescence in *L. perenne* varieties grown under long term exposure to shade. The authors measured minimum fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), and maximum photosynthetic efficiency of photosystem II (F_v/F_m). Prokopiuk *et al.* (2019) investigated the effects of fabric coverings on natural grass sports pitches, which help to protect turfgrass surfaces during the winter period, and use fluorescence parameters minimum fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), and calculated maximum photosynthetic efficiency of photosystem II (F_v/F_m).

2.2.4 Fluorescence measurements used in this study

Using dark-adapted and light-adapted measurements, Murchie and Lawson (2013) recommend using three key chlorophyll fluorescence parameters:

a. PSII operating efficiency (*ØPSII* or *Fq'/Fm'*)

The operating efficiency of PSII photochemistry gives the proportion of absorbed light that is actually used in PSII and can be used to estimate the rate of electron flow through PSII, measured with the parameters *Fq'/Fm'*

(Murchie and Lawson, 2013). Chlorophyll fluorescence measurements of the operating efficiency of PSII (ØPSII) were performed on dark adapted turfgrass species of *L. perenne* grown in 3 ½ inch pots.

ØPSII is the simplest fluorescence parameter to measure requiring just pointing a fluorometer at a leaf and flashing a light (Maxwell and Johnson, 2000). PSII activity is sensitive to abiotic and biotic factors and is a key technique for understanding how plants respond to environmental changes (Murchie and Lawson, 2013). Measured values for ØPSII vary between 0 and 1 (Baker 2008) but, due to the inherent inefficiencies of PSII, values vary from zero to the F_v/F_m values of 0.83-0.85 (Kalaji *et al,.* 2014). Values decrease as photosynthetic photon flux density (PPFD in μmol m^{-2} s^{-1}) increases due to limitations for the flow of electrons on the acceptor side of PSII (Kalaji *et al.,* 2014). Environmental stresses decrease stomatal conductance (entry of CO_2 into the leaf), carbon metabolism and transport processes, which can also decrease PSII efficiency (Baker, 2008). F_q'/F_m' is a very useful measurement to use in the field but if used to calculate Electron Transport Rate (ETR) the interpretation of any changes in ETR values needs to take into consideration that ETR is highly dependent on ambient light levels and measuring PAR at the leaf surface at the same time as F_q'/F_m' is vital for accuracy (Murchie & and Lawson 2013). ETR is calculated using the formula;

$$\text{ETR} = \Phi\text{PSII} \text{ x } 0.5 \times 0.84$$

where 0.5 is a factor that assumes 50% partitioning of energy to each of PSII and PSI, and 0.84 is an assumption of the % of PAR absorbed at the leaf surface (Murchie and Lawson, 2013). ETR has not been calculated in this study due to unknown variables in the leaf absorption rate and division of energy in turfgrass plants when under different stresses. More work is required in this area.

b. Fraction of open PSII centers, qL (Lake Model)

qL (Lake Model) measures the fraction of PSII reaction centres that are open, i.e. the fraction of PSII reaction centres with Q_A in the light-adapted state that are fully oxidised and capable of performing photochemical reduction (Kalaji *et al.* 2017). The *qL* lake model assumes that an individual antenna pigment can transfer absorbed energy to any of the neighbouring reaction centres embedded in a network of antenna pigments (Gu *et al.* 2019). Values near zero indicate that Q_A is in the reduced state and most of the PSII reaction centres are closed. A reduced rate in the electron transport pathway from PSII to PSI, may be caused by a sudden increase in light that diminishes the reducible pool of the primary quinone electron acceptor, or by decreased consumption of ATP and NADPH energy due to limited CO_2 supply for the dark reactions under environmental stresses. The D1 protein of the PSII reaction centre can also degenerate under sustained increases in light, leading to its inactivation and a decrease in qL. More closed reaction centres lead to a reduction in PSII efficiency and an increase in fluorescence and NPQ (Maxwell and Johnson 2000; Kalaji *et al.* 2017). For the parameter qL, the greater the value the greater the number of open reaction centres (Gonzales *et al.* 2012).

Increases in PAR also decreases qL (Gu et.al, 2019). The equation used is $(F_q'/F_v')/F_0'/F_{'})$ (Murchie and Lawson 2013).

c. Non-Photochemical Quenching (NPQ),

Non-Photochemical Quenching (NPQ) quantifies the regulated process in which leaves dissipate excess absorbed photon energy into harmless heat, relative to the dark-adapted state, and is the most important protective mechanism of PSII employed by plants (Gu *et al*. 2019). Most plants receive more sunlight than they can use in photosynthesis (Müller *et al*. 2001) and NPQ acts as a safe mechanism that removes excess excitation energy within chlorophyll containing complexes to prevent the likelihood of the formation of plant damaging free radicals such as singlet oxygen ($^1O_2*$) (Murchie and Lawson 2013 and Müller *et al*. 2001). Theoretical values for NPQ range from zero to infinity (Kalji *et al.* 2014) but in most cases range from 0.5 – 3.5 in a typical plant. Changes in NPQ is a measurement of changes in the efficiency of heat dissipation resulting from processes that protect the leaf from light-induced damage or of the damage itself (Maxwell and Johnson 2000). High light levels cause an increase in the electron transport rate and results in acidification of the thylakoid lumen, the enzyme violaxanthin is converted to zeaxanthin which is an efficient quencher of excitation energy in the PSII antenna, and an increase in heat loss occurs (Baker 2008). NPQ values can be low during continuous or extreme stress that can lead to a loss of reaction centres (Kalaji *et al.* 2014).

d. Maximum Quantum Yield of PSII, (F_v/F_m)

To the above list I have added Maximum Quantum Yield of PSII, or F_v/F_m, which is a robust indicator of the maximum quantum yield of PSII chemistry (Murchie and Lawson, 2013), and a parameter commonly used to indicate plant stress. F_v/F_m is interrelated to ØPSII and *qL and* measures the maximum efficiency (quantum yield) of PSII, i.e. the quantum efficiency if all PSII centres were open. Dark adapted measurements are used as a sensitive indicator of plant photosynthetic performance, with a value of 0.83-0.84 being the optimum for most C_3 plant species (Kalaji *et al.* 2014). A change in F_v/F_m is due to a change in NPQ (Murchie and Lawson 2013) and plants showing values lower than 0.83 indicate they have been exposed to abiotic and/or biotic stress in the light. F_v/F_m provides a simple and rapid method of stress monitoring in plants (Maxwell and Johnson 2000, Baker 2008), but care should be taken in interpreting the results as stress to other parts of the plant (e.g. roots), and which affect plant health and growth, may not be measured by F_v/F_m (Murchie and Lawson 2013). F_v/F_m values can also be underestimated in heat stress conditions due to a loss of electron donation capacity and not to a change in PSII quantum yield (Kalaji *et al.* 2017; Lawson 2013).

Table 2 summarises the fluorescence parameters used in this study.

Table 2. Chlorophyll fluorescence imaging parameters used in this study (Baker 2008; Murchie and Lawson 2013; Cendrero-Mateo *et al.* 2015)

Parameter	**Definition**
ØPSII *(Fq'/Fm')*	Measures the operational efficiency of PSII in light after dark adaptation. Calculated by dividing the number of molecules undergoing the process by the number of photons absorbed by the system, with an efficiency of 1.0 (100%) being the maximum possible value.
Maximum Quantum Efficiency *(Fv/Fm)*	Maximum efficiency at which light absorbed by PSII is used for reduction of Q_A (photochemistry) (Cendrero-Mateo *et al* 2015). An Fv/Fm value in the range of 0.79 to 0.84 is the approximate optimal value for many plant species, with lower values indicating plant stress (Maxwell K., Johnson G. N. 2000).
qL	Estimates the fraction of open PSII centres
Non-Photochemical Quenching *(NPQ)*	A process in which excess absorbed light energy is dissipated into heat. Occurs when there is an increase in the rate at which excitation within Photosystem II is lost as heat.

In summary, in treated and non-treated samples subject to full daylight and 50% daylight:

- *ØPSII* relates to efficiency of photosynthetic processes
- *qL* and F_v/F_m providing information about the underlying processes that have altered ØPSII
- *NPQ* measures a change in heat dissipation, relative to the dark-adapted state, and helps to protect PSII process from light-induced damage (Maxwell and Johnson, 2000).

2.2.4.1 Method of measuring chlorophyl fluorescence parameters

The pots were placed into a FluorCam FC800-222 (Photon Systems Instruments, Drasov, Czech Republic) 0, 2, 4, 6, 8 and 10 days after treatment (Figs 22).

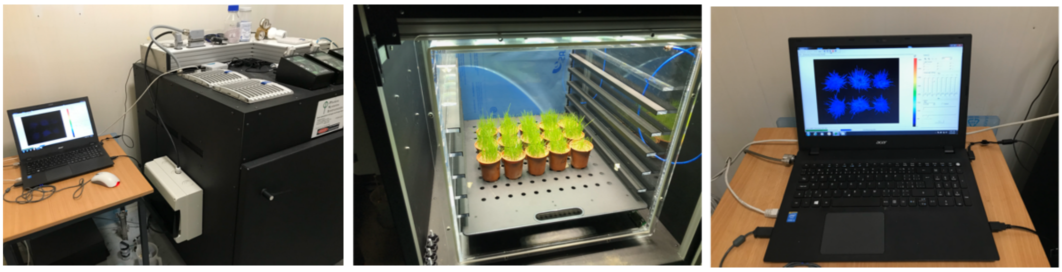


Fig 22. FluorCam FC800-222. L-R: Fluorcam connected to PC, plant samples inserted in the chamber, computer interface for the Fluorcam

The FluorCam contains a CCD camera that captures images, modules that control the measuring light flashes called actinic light and saturating flashes that are generated by the light sources. (Fluorcam Operating Instructions - https://fluorcams.psi.cz/documents/FluorCam_Operation_Manual_2.1.pdf).

The pots were dark adapted for one hour inside the Fluorcam and then subjected to the following experiment parameters:

Dark adaptation: 1 hour
Protocol used: LRC (Actinic 2)
Shelf No. 2

Settings
UV=0
FAR=0
LightA=0
Light B=0
Light Intensity=<10,20,40,60,80,100)
Super=80
Act2=0
Act1=0
Sensitivity=13.4 (varied up to 17 depending on stage of plant growth)
Shutter=0

PPFD Intensities for the LRC

ID	%	PPFD
LSS1	10	21.71
LSS2	20	141.81
LSS3	40	407.7
LSS4	60	664
LSS5	80	903.7
LSS6	100	1148.3

2.2.5 Statistics

Light curves for days 2, 4, 6, 8 and 10 after treatment with 100mg L^{-1} were generated for ØPSII, qL and NVQ. Line charts were generated to present Fv/Fm over the 10-day period. The mean measurements of Control Light, Treated Light, Control Shade and Treated Shade at each PPFD point (Lss 1-6) within each Day After Treatment were compared using ANOVA followed by Duncan's multiple range test, a post hoc test that measures specific differences between pairs of means, with level of significance $P \leq 0.05$. Measurements between days were not subjected to statistical analysis. Statistical analysis was carried out with statistical software: VSN International (2020). Genstat *for Windows* 21st Edition. VSN International, Hemel Hempstead, UK. Web page: www.genstat.co.uk. The Null Hypothesis was that exogenous applications of 5-ALA do not increase fluorescence values. The alternative Hypothesis is that exogenous applications of 5-ALA increase fluorescence values.

Chapter Three - Results

3.1 Experiment One Results

3.1.1 % Dry Weight

Full results and statistical analysis can be found in Appendix II. Fig 23 presents the findings for % dry weight of leaf clippings on each sample day after treatment.

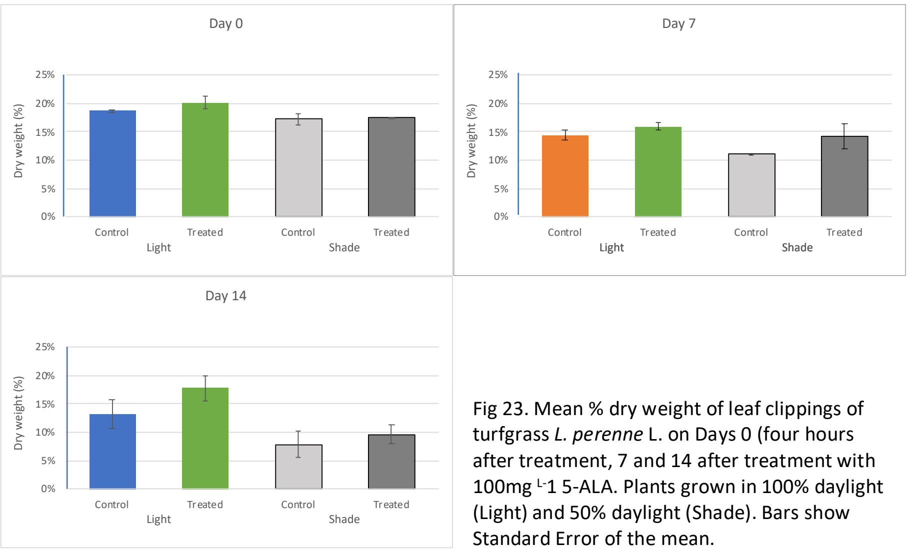


Fig 23. Mean % dry weight of leaf clippings of turfgrass *L. perenne* L. on Days 0 (four hours after treatment, 7 and 14 after treatment with 100mg $^{L-}$1 5-ALA. Plants grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.

Significant lower % Dry Weights occurred on Day 0 (four hours after treatment) between plots grown in 100% or 50% daylight (P=0.027), and on Day14 between plots grown in 100% or 50% daylight (P=0.015).

These results show that treatments with 100mg L^{-1} 5-ALA did not have significant effects on % Dry Weight of turfgrass *L. perenne* grown in 100% and 50% daylight.

3.1.2 Chlorophyl Index (NDVI)

Full results and statistical analysis can be found in Appendix III. Fig 24 presents the findings of measured Chlorophyll Index values of turfgrass plants on each sample day after treatment.

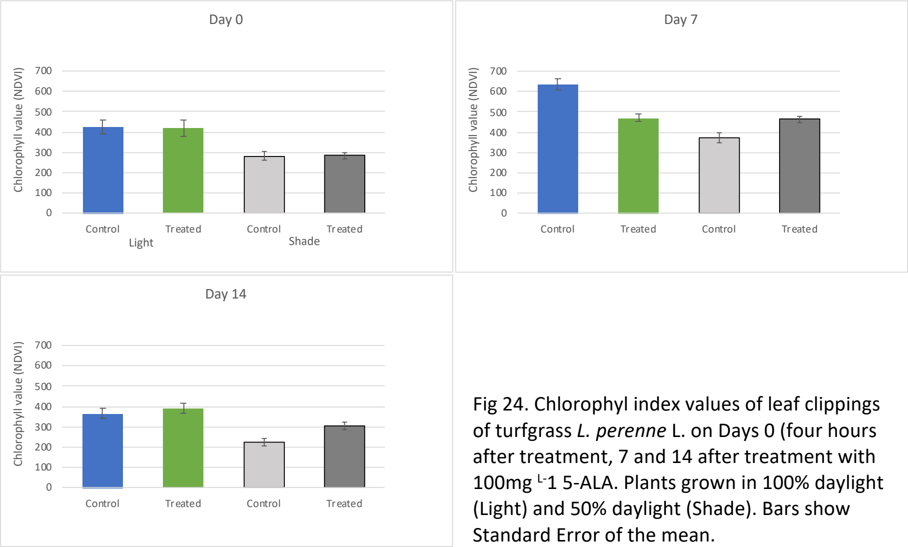


Fig 24. Chlorophyl index values of leaf clippings of turfgrass *L. perenne* L. on Days 0 (four hours after treatment, 7 and 14 after treatment with 100mg $^{L-}$1 5-ALA. Plants grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.

Significantly lower Chlorophyll Index values were observed on Day 0 (four hours after treatment) in plots grown in 50% daylight (P<0.001).

On Day 7, Treated Light plots had significantly lower Chlorophyll Index values than the Control due to 5-ALA treatments (P<0.001), and Treated Shade plots had significantly higher NDVI values than Control Shade due to 5-ALA treatments (P<0.001). There was no significant difference between Treated Light and Treated Shade plots. Plots grown in 100% Daylight had significantly higher NDVI values than plots grown in 50% Daylight (P<0.001).

Chlorophyll Index values in 50% Daylight on Day 14 after treatment showed significant increase due to 5-ALA treatments (P=0.024) in Treated plots compared to Control plots, but no difference in 100% Daylight plots between treated and non-treated. Plots grown in 100% daylight showed significantly higher NDVI values (P<0.001) than plots grown in 50% daylight.

These results show that on Days 7 and 14 after treatment with 100mg L^{-1} on turfgrass *L. perenne* L., 5-ALA lowered Chlorophyll Index values in plants grown in 100% Daylight but increased Chlorophyll Index values in plants grown in 50% Daylight.

3.1.3 Leaf nutrient content

A full nutrient analysis was carried out using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Full results and statistical analysis can be found in Appendix IV. Figs. 25-38 show the effects in chart form for each nutrient tested for each sample day after treatment.

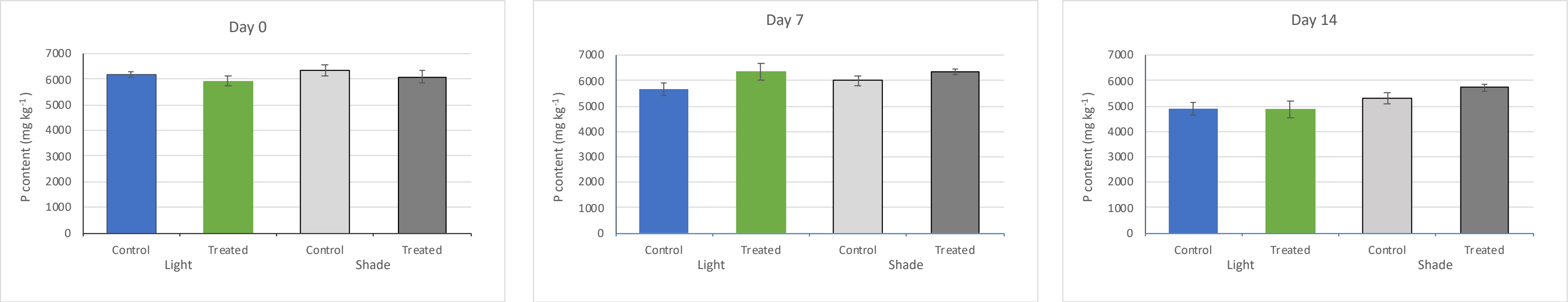


Fig. 25. P content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

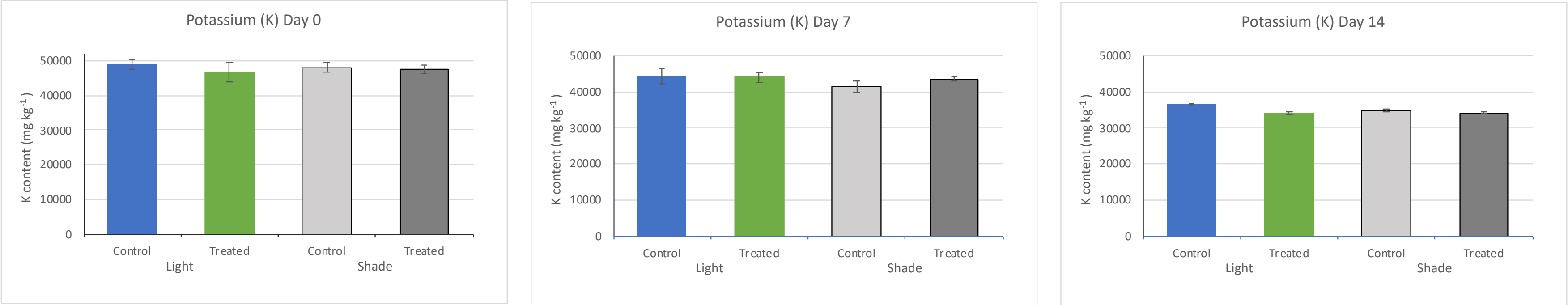


Fig. 26.K content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

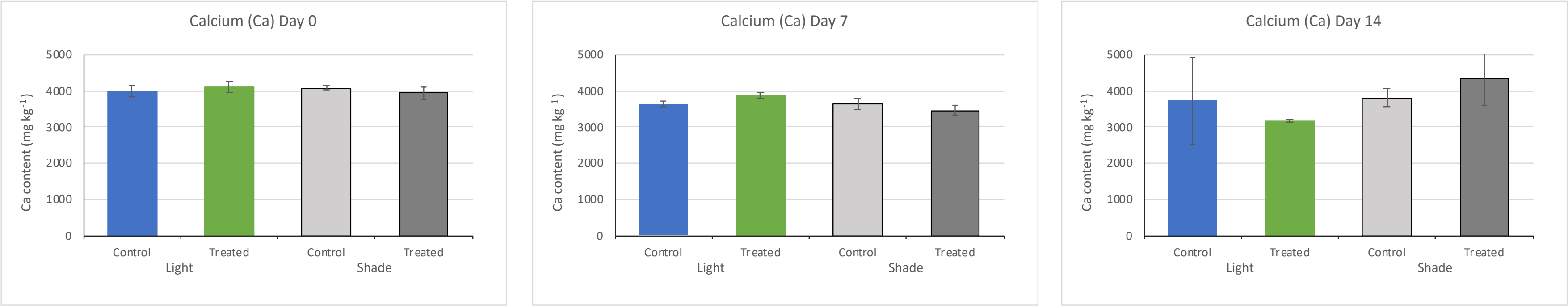


Fig. 27. Ca content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

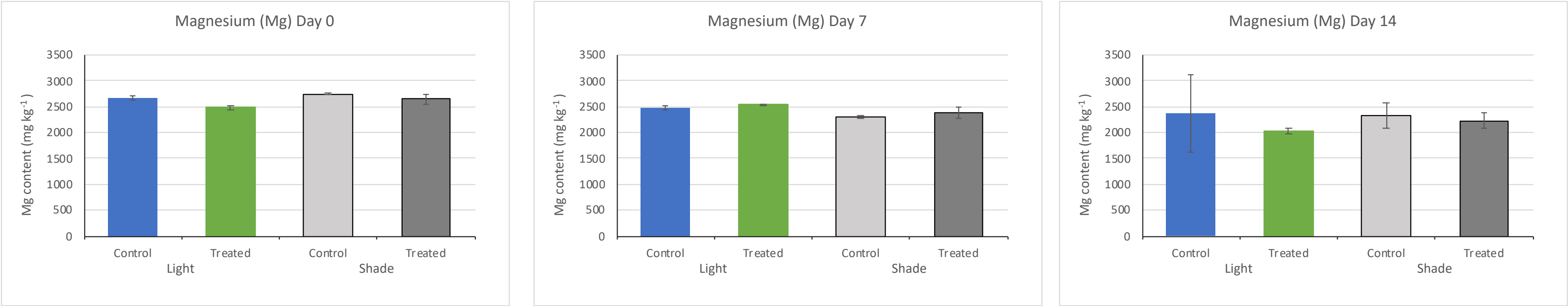


Fig. 28. Mg content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

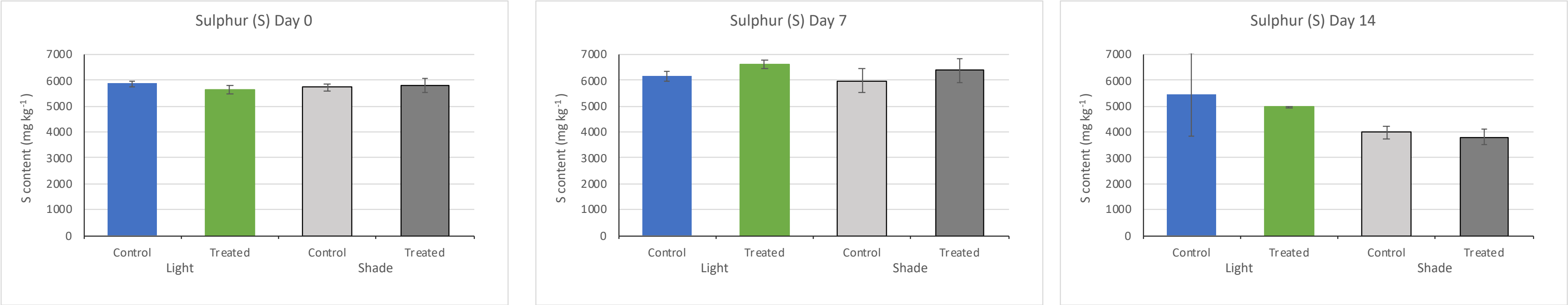


Fig. 29. S content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

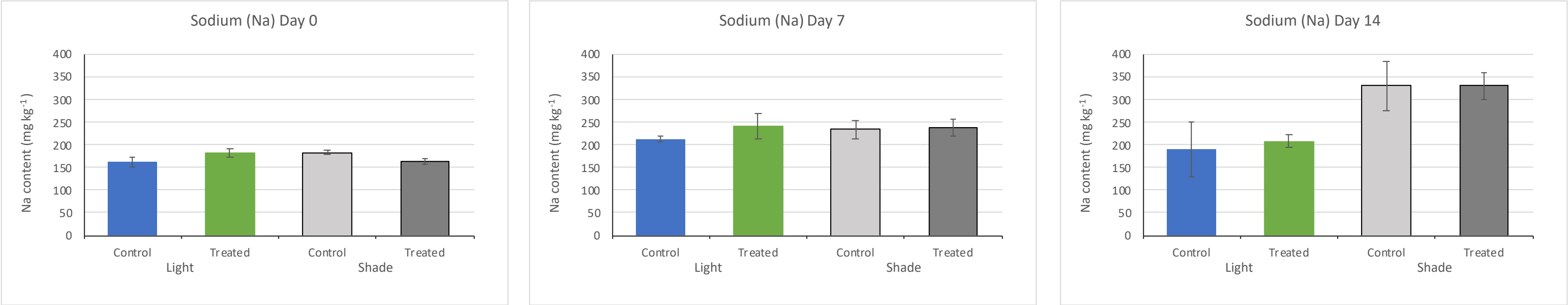


Fig. 30. Na content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

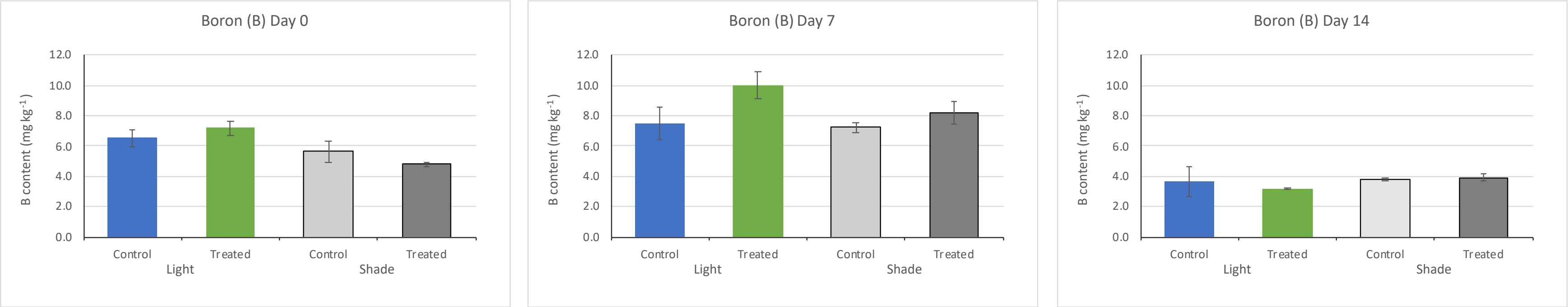


Fig. 31. B content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

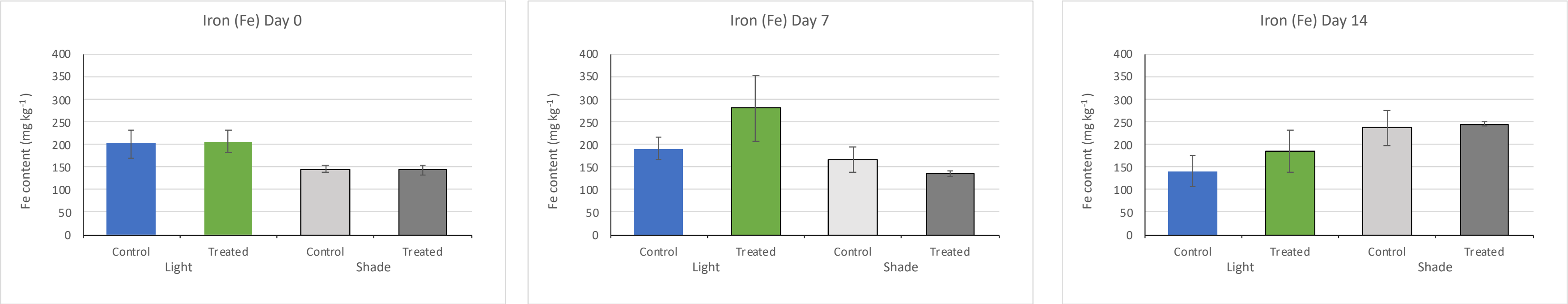


Fig. 32. Fe content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

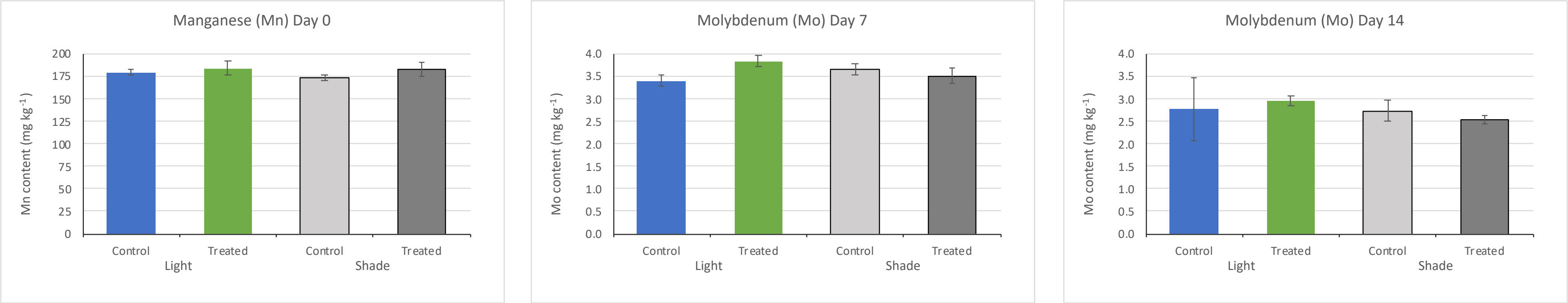


Fig. 33. Mn content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

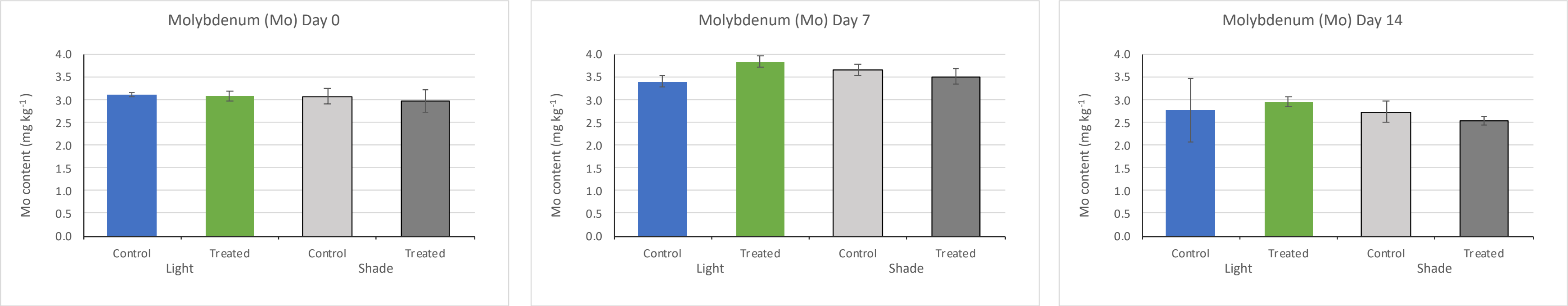


Fig. 34. Mo content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

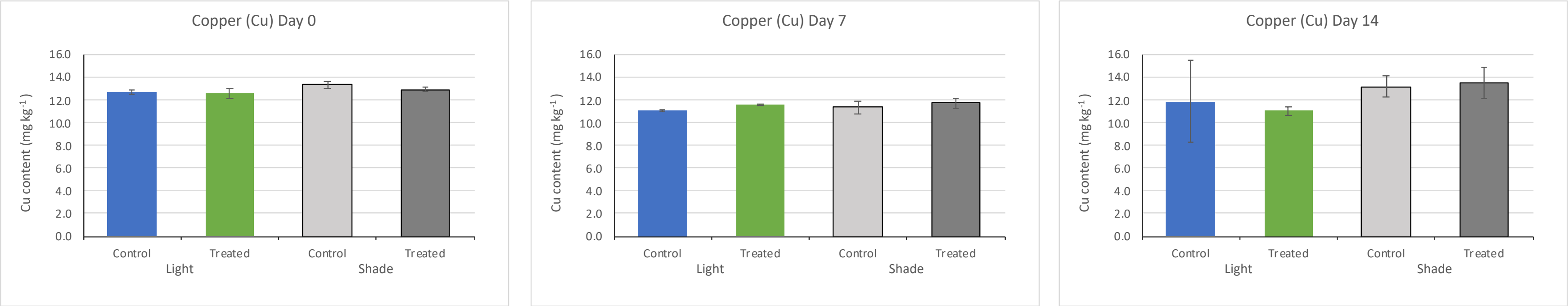


Fig. 35. Cu content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

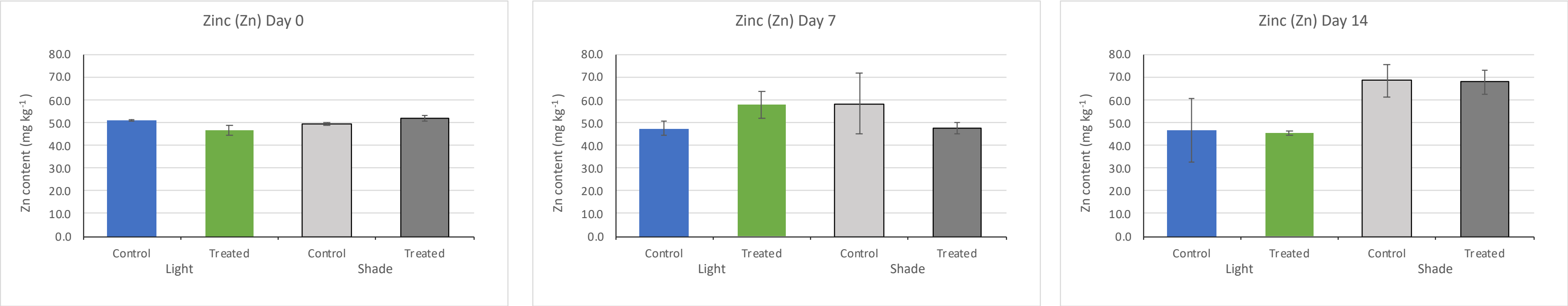


Fig. 36. Zn content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

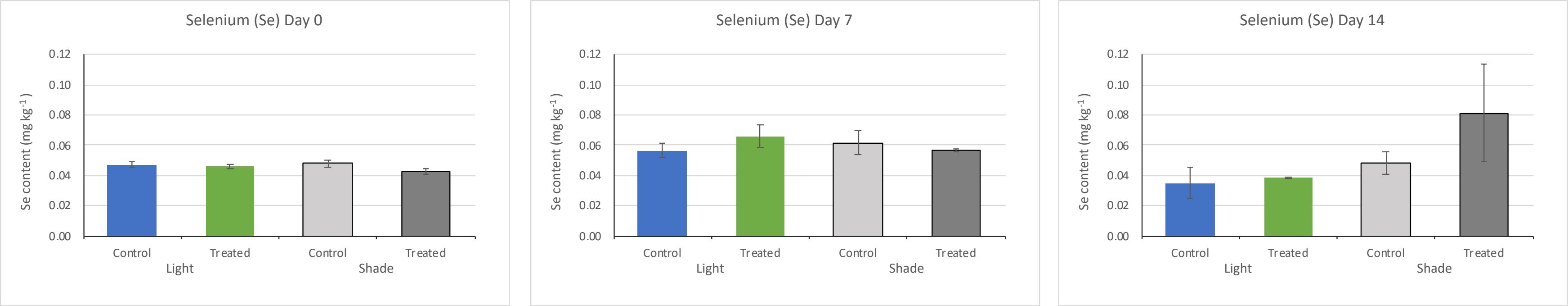


Fig. 37. Se content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

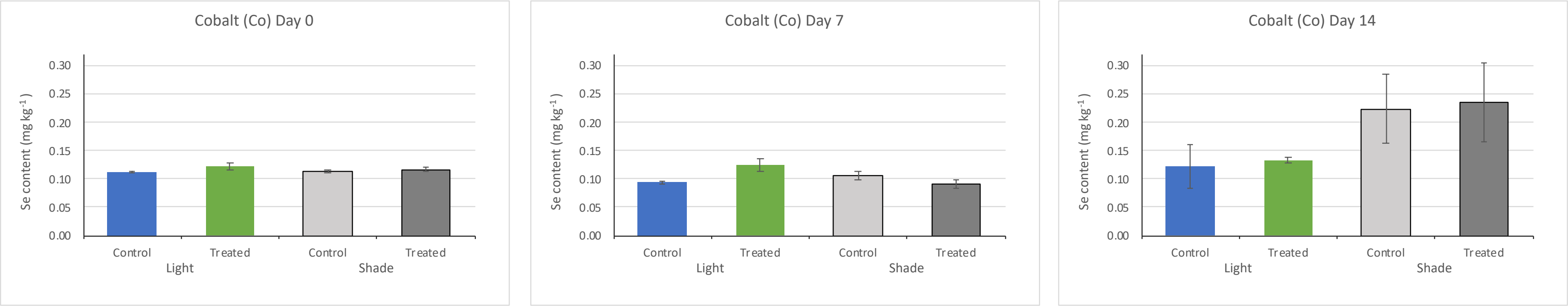


Fig. 38. Co content (mg kg^{-1}) in turfgrass leaves (*Lolium perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade). Charts show Days after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Significant differences (P ≤0.05) occurred in leaf content of the following nutrients:

a) Magnesium Mg – on Day 0 (four hours after treatment) Treated plants grown in 100% daylight had a lower Mg content (P=0.035) due to treatment with 5-ALA compared to the Control Treated plants grown in 50% daylight. On day 7 after treatments Light grown plants showed significantly higher (P=0.024) calcium content than in Shade grown plants.

b) Sodium (Na) – on Day 0 (four hours after treatment) Treated plants grown in 100% daylight had a significantly higher (P=0.035) Na content than the Control due to 5-ALA treatments, and Treated plants grown in 50% daylight had a significantly lower (P=0.035) Na content than the Control Shade due to 5-ALA treatments. On Day 14 after treatments Shade grown plants had significantly higher Na content than Light grown plants (P=0.017).

c) Boron (B) – on Day 0 (four hours after treatment) plants grown in 100% daylight showed significantly higher (P=0.013) B content than plants grown in 50% daylight.

d) Iron (Fe) – on Day 0 (four hours after treatment) plants grown in 100% daylight showed significantly higher (P=0.025) B content than plants grown in 50% daylight.

e) Manganese (Mn) – on Day 7 after treatments Treated plants grown in 100% daylight had a higher Mn value (P=0.013) than Light Control plants due to 5-ALA treatments. There were no significant differences between Treated and Control plants grown in 50% daylight.

f) Zinc (Zn) – on Day 0 (four hours after treatment) Treated plants grown in 50% daylight shown the highest Zn content (P=0.027) due to 5-ALA treatments. There are also significant differences (P=0.027) between plants grown in 100% and 50% daylight. On Day 14 after treatments there were significantly higher (P=0.027) leaf Zn content in plants grown in 50% daylight than in plants grown in 100% daylight.

g) Cobalt (Co) – on Day 7 after treatments Treated plants grown in 100% daylight had higher (P=0.013) Co content than the Control. Plants grown in 100% daylight had significantly higher (P=0.013) Co content than plants grown in 50% daylight.

3.2 Experiment Two Results

Chlorophyll a fluorescence

The full set of results and statistical analysis for chlorophyl fluorescence can be found in Appendix V. During the period of the experiment the turfgrass plant samples experienced higher than optimum range of solar irradiance and air temperature for healthy turfgrass growth. The impact of these measurements is discussed in Chapter 4 - Discussion.

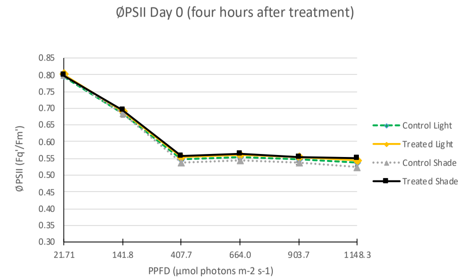
Fig 39 presents the light response curves on each of the sampling days after treatment for:

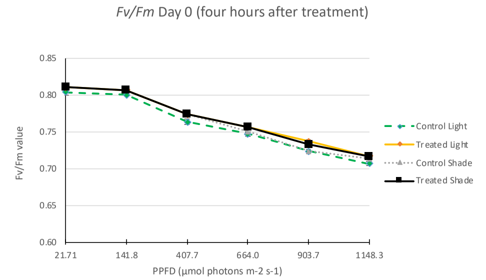
i. ØPSII operating efficiency (F_q'/F_m')
ii. F_v/F_m (maximum quantum efficiency of PSII photochemistry)
iii. *qL* (fraction of PSII centres that are open)
iv. NPQ (non-photochemical quenching)

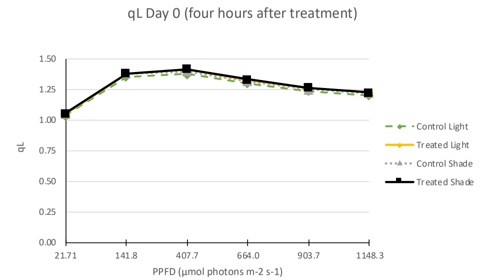
i. *PSII Operating Efficiency (ØPSII or F_q'/F_m')*

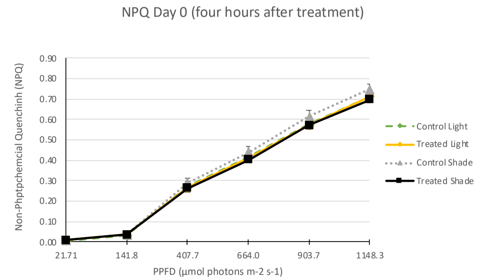
Fig. 39 presents the measurements of *ØPSI* observed on days 0, 2, 4, 6, 8 and 10 after treatment in this experiment.

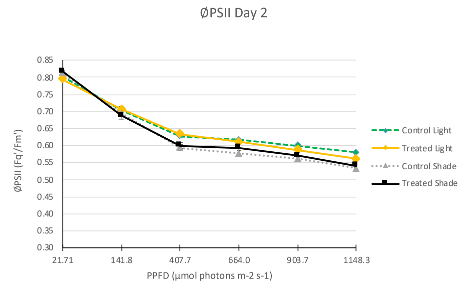
The general trend showed that ØPSII decreased with increasing PPFD irradiance from PPFD 21.7 to 407.7, followed by a less steep decline in values. Values for ØPSII in Light were highest on Day 2 for both Control and Treated

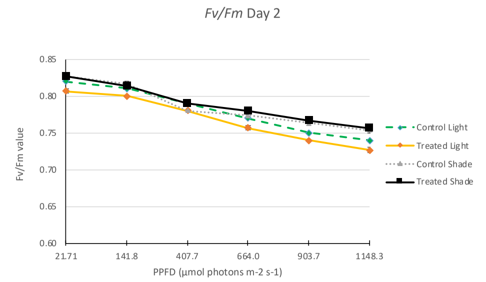

ØPSII Day 0 (four hours after treatment)

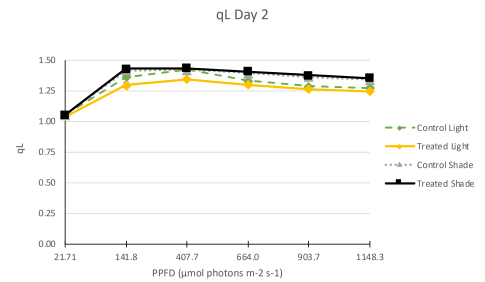

Fv/Fm Day 0 (four hours after treatment)

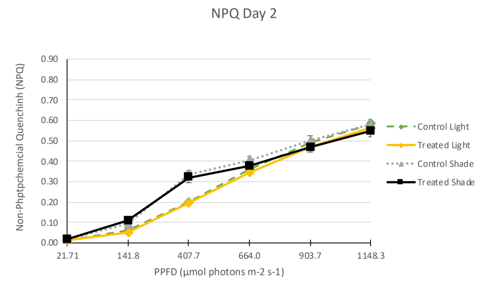

qL Day 0 (four hours after treatment)

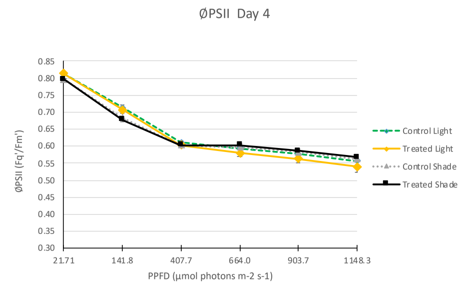

NPQ Day 0 (four hours after treatment)

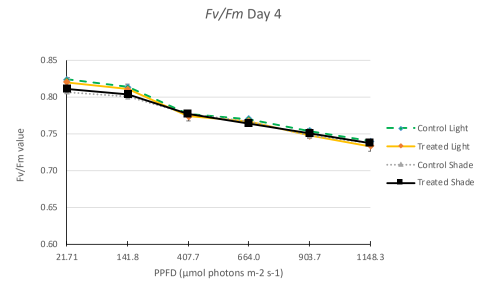

ØPSII Day 2

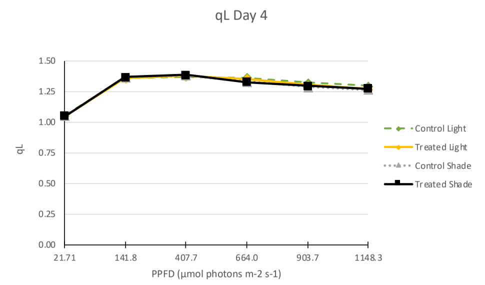

Fv/Fm Day 2

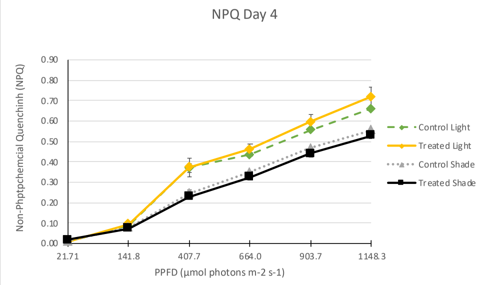

qL Day 2


NPQ Day 2


ØPSII Day 4


Fv/Fm Day 4


qL Day 4


NPQ Day 4

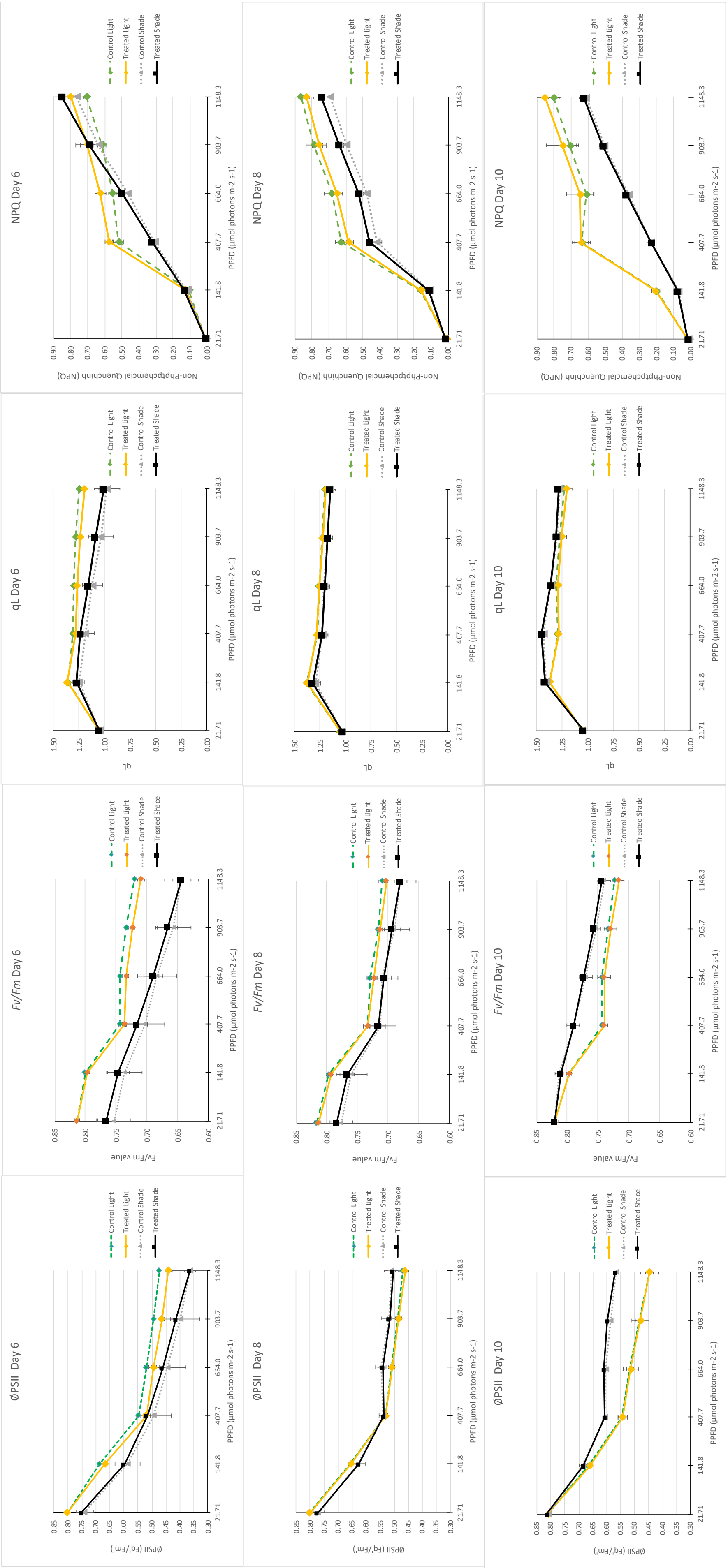


Fig 39. Effects of treatment with 100mg L^{-1} 5-aminolevulinic acid on ØPSII, F_v/F_m, qL and NPQ in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade). Measurements at each PPFD were compared for each treatment. PPFD = Photosynthetically active Photon Flux Density. Bars show Standard Error of the Mean

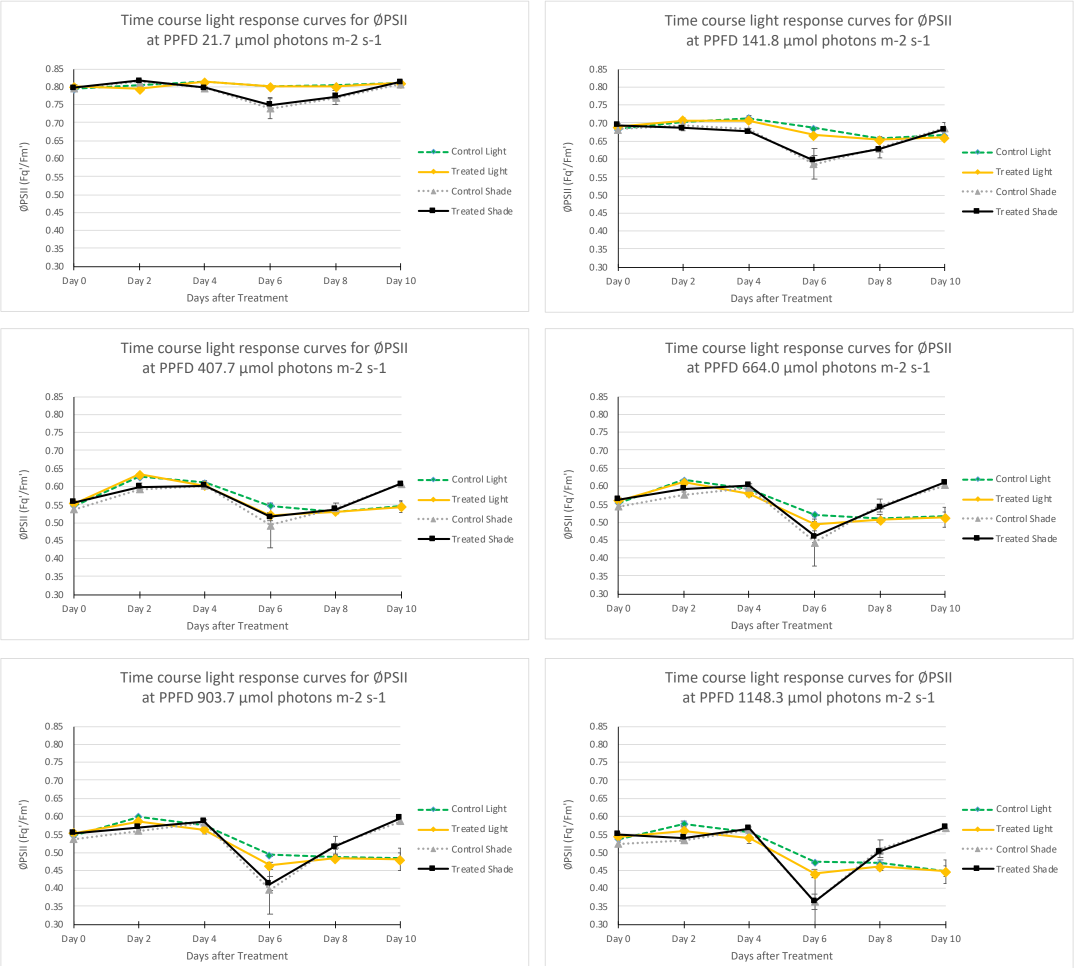
samples. Light response was highest in Shade in both Control and Treated shade on Day 10, with lowest measurements on Day 6.

Significant differences due to treatments of 5-ALA only occurred at 1148.3 µmol photons m^{-2} s^{-1} (100%) on Day 0 (four hours after treatment) between Control and Treated in 50% daylight ($P=0.01$).

Significant difference occurred between plants grown in Light and Shade on:

- Day 2 PPFD µmol photons m^{-2} s^{-1}: 21.71 ($P=0.001$), 407.7 ($P=0.002$), 664.0 ($P=0.002$), 903.7 ($P= 0.005$) and 1148.3 ($P=0.003$)
- Day 4 PPFD µmol photons m^{-2} s^{-1}: 21.71 ($P=0.001$), 141.8 ($P=0.005$) and 664.0 ($P=0.05$)
- Day 6 PPFD µmol photons m^{-2} s^{-1}: 21.71 ($P=0.013$), 141.8 ($P=0.006$) and 1148.3 ($P=0.03$)
- Day 8 PPFD µmol photons m^{-2} s^{-1}: 664.0 ($P=0.013$), 903.7 ($P=0.046$) and 1148.3 ($P=0.027$)
- Day 10 PPFD µmol photons m^{-2} s^{-1}: 141.8 ($P=0.029$), 407.7 ($P<0.001$), 664.0 ($P<0.001$), 903.7 ($P<0.001$) and 1148.3 ($P=<0.001$).

The Time Response Curves in Fig 40 show a general increase in ØPSII values from Day 0 to Day 2 for Control and Treated plants grown in Shade and Light, followed by a decline, especially in Shade plants, to Day 6. ØPSII in Shade grown plants recovered steeply and continued to rise by Day 10.


Time course light response curves for ØPSII at PPFD 21.7 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 141.8 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 407.7 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 664.0 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 903.7 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 1148.3 µmol photons m-2 s-1

Fig 40. Time course response of ØPSII at each PPDF over the period of the experiment (days after treatment) of turf type *Lolium perenne* (L.) to treatments with 100mg L^{-1} 5-Aminolevulinic acid, applied to plants grown in 100% Daylight (Light) and 50% Daylight (Shade). The chlorophyll fluorescence parameter F_v/F_m was measured on days 0, 2, 4, 6, 8 and 10 after treatments. PPFD = Photosynthetically active Photon Flux Density. Bars show Standard Error of the Mean.

Light grown plants showed a slow recovery after Day 6 at PPDF 407.71 and 664.0 but continued to decline at a slower rate at PPDF 903.7 and 1148.3. The statistical analysis shows that these differences were due to whether the plants were grown in Light or Shade. The high temperatures in the glasshouse during this period may have caused inhibition of photosynthesis, especially in plants grown in Shade. Cooler temperatures will have resulted in increased CO_2 uptake and an increase in ØPSII, but also allowed the high light to cause more stress on the plants grown in 100% daylight.

5-ALA treatments had a significant effect on ØPSII only on Day 0 in Shade, with values in Treated plants 5.5 % more than the non-treated Control (P=0.01). This value was not significantly different than Control and Treated Light plants, indicating that 5-ALA treatments could benefit grass grown in shaded areas for a short period of time after treatment.

ii. Maximum Quantum Yield of PSII Photochemistry (Fv/Fm)

Figs. 39 presents the measurements of F_v/F_m observed on days 0, 2, 4, 6, 8 and 10 after treatment in this experiment.

The general trend showed that F_v/F_m values decreased with increasing irradiance. A steep decline in values from PPFD 141.8 to 407.7 occurred in Light grown plants (treated and control) on days 6, 8 and 10, followed by a less steep decline in values. This also occurred in Shade grown plants on day 8 after treatments. Significant differences between Control and Treated plants from treatments of 5-ALA occurred:

- Day 0 PPFD µmol photons m^{-2} s^{-1}: 664.0 between Control and Treated in plants grown 100% daylight (P=0.020), and at 903.7 (P=0.008).
- Day 2 PPFD µmol photons m^{-2} s^{-1}: between Control and Treated in plants grown 100% daylight, 27.1 (P=0.050), and 141.8 (P=0.022)

Significant differences between plants grown in 100% and 50% daylight, and non-treated and treated with 5-ALA occurred:

- Day 2 PPFD 407.7 µmol photons m^{-2} s^{-1} (P<0.001), PPFD 664.0 µmol photons m^{-2} s^{-1} (P=0.003), PPFD 903.7 µmol photons m^{-2} s^{-1} (P=0.022), and at 1148.3 PPFD µmol photons m^{-2} s^{-1} (P=0.020)

Significant difference occurred between plants grown in 100% daylight and 50% daylight on:

- Day 2 PPFD µmol photons m^{-2} s^{-1}: 141.8 (P=0.003), 664.0 (P<0.001), 903.7 (P<0.001) and 1148.3 P<0.001)
- Day 4 PPFD µmol photons m^{-2} s^{-1}: 21.71 (P<0.001), 141.8 (P=0.003)
- Day 6 PPFD µmol photons m^{-2} s^{-1}: 21.71 (P=0.008), 141.8 (P=0.010), 664.0 (P=0.20), 903.7 (P=0.004) and 1148.3 (P=0.002)
- Day 8 PPFD µmol photons m^{-2} s^{-1}: 21.71 (P=0.011)
- Day 10 PPFD µmol photons m^{-2} s^{-1}: 141.8 P<0.006), 407.7 (P<0.001), 664.0(P<0.004), 903.7 (P=0.007) and 1148.3 (P=<0.015).

Figure 41 presents the time course response for each PPDF used for the fluorescence for each of the sampling days after treatment. A steep decline in F_v/F_m values for Control and Treated plants grown in Shade between Days 4 and 6 after treatment, followed by a steep recovery to Day 10. The statistical analysis shows that these differences were significant due to whether the plants were grown in Light or Shade.

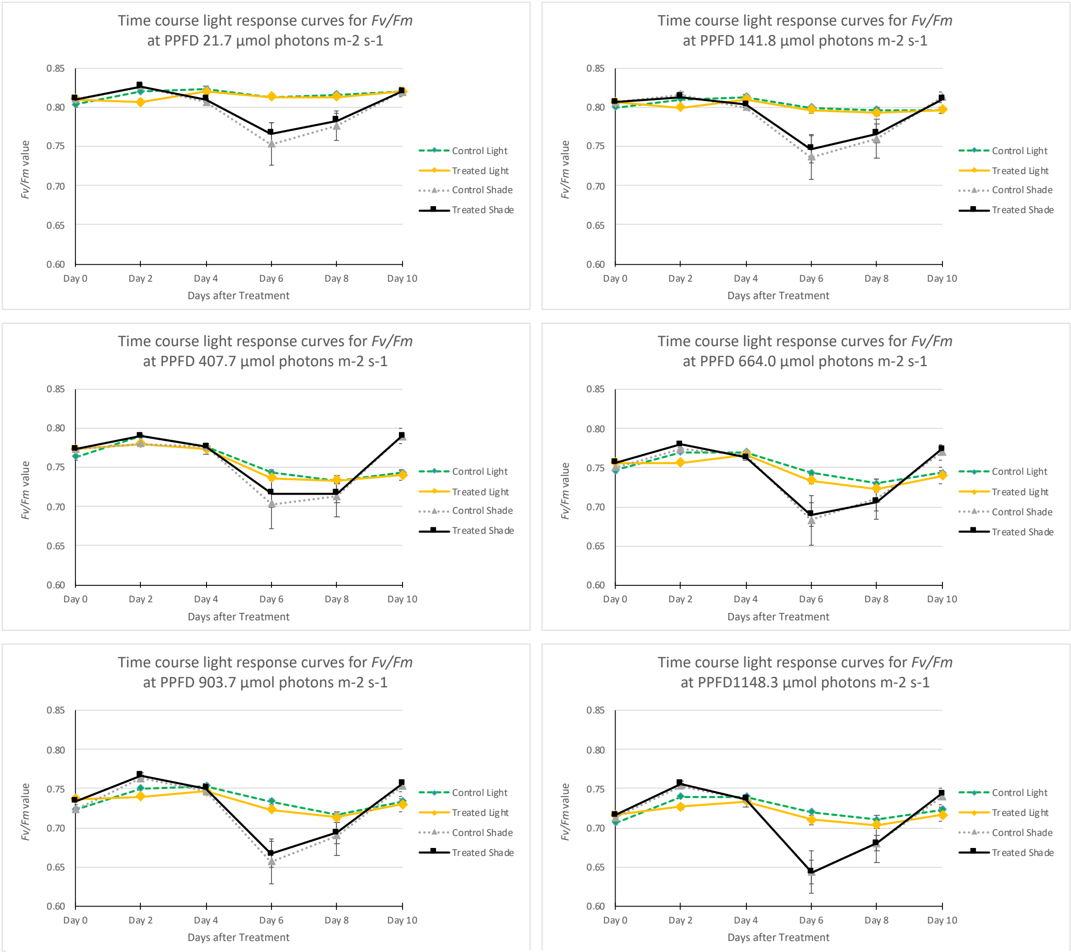


Fig 41. Response for Fv/*Fm* at each PPDF over the period of the experiment (days after treatment) of turf type *Lolium perenne* (L.) to treatments with 100mg L^{-1} 5-Aminolevulinic acid, applied to plants grown in 100% Daylight (Light) and 50% Daylight (Shade). The chlorophyll fluorescence parameter F_v/F_m was measured on days 0, 2, 4, 6, 8 and 10 after treatments. PPFD = Photosynthetically active Photon Flux Density. Bars show Standard Error of the Mean.

The high air temperatures in the glasshouse during this period (over 40^{0}C) may have caused restricted uptake of CO_2, especially in plants grown in 50% daylight. Cooler temperatures will have resulted in increased CO_2 uptake, but also allowed the high light to cause more stress on the plants grown in 100% daylight.

iii. qL (Estimate of the fraction of open PSII centres)

Fig. 39 shows the qL for days 0 (four hours after treatment), 2, 4, 6, 8, and 10 after treatment with 5-aminolevulinic acid. There were significant differences on Day 0 and Day 2 due to treatments of 5-ALA between the Control and Treated:

- On Day 0 (four hours after treatment) a significantly lower value in Control Light was found at 21.7 µmol photons m^{-2} s^{-1} (P=0.05), in Control Light at 903.7 µmol photons m^{-2} s^{-1} (P=0.04), and at 1148.3 µmol photons m^{-2} s^{-1} (P=0.032)
- On Day 2 a significantly lower qL value was found in Treated Light at 407.7 µmol photons m^{-2} s^{-1} (P=0.004).
- Significant differences in qL due to a combination of 5-ALA treatments and plants grown in 100% and 50% daylight were observed at 407.7 (P<0.001), 664.0 (P=0.011), 903.7 (P=0.028) and 1148.3 (P<0.029). µmol photons m^{-2} s^{-1}.

Significant differences in qL between plants grown in Light and Shade were found on:

- Day 0 at 21.7 µmol photons m^{-2} s^{-1} (P=0.009)
- Day 2 at µmol photons m^{-2} s^{-1} 141.8 (P=0.001), 407.7 (P<0.001), 664.09 (P<0.001), 903.7 (P<0.001). and at 1148.3 µmol photons m^{-2} s^{-1} (P<0.001)
- Day 4 at 664.0 µmol photons m^{-2} s^{-1} (P=0.041)
- Day 6 at 141.8 µmol photons m^{-2} s^{-1} (P=0.017), 141.8 (P=0.017), 664.0 (P=0.03), 903.7 (P=0.015), and 1148.3 (P=0.009)

- Day 8 at 21.7 µmol photons m^{-2} s^{-1} (P=0.01)
- Day 10 at µmol photons m^{-2} s^{-1} 141.8 (P<0.001), 407.7 (P<0.001), 664.09 (P=0.022).

Figure 42 presents the Time Response Curve at each PPDF used for the fluorescence for each of the days after treatment.

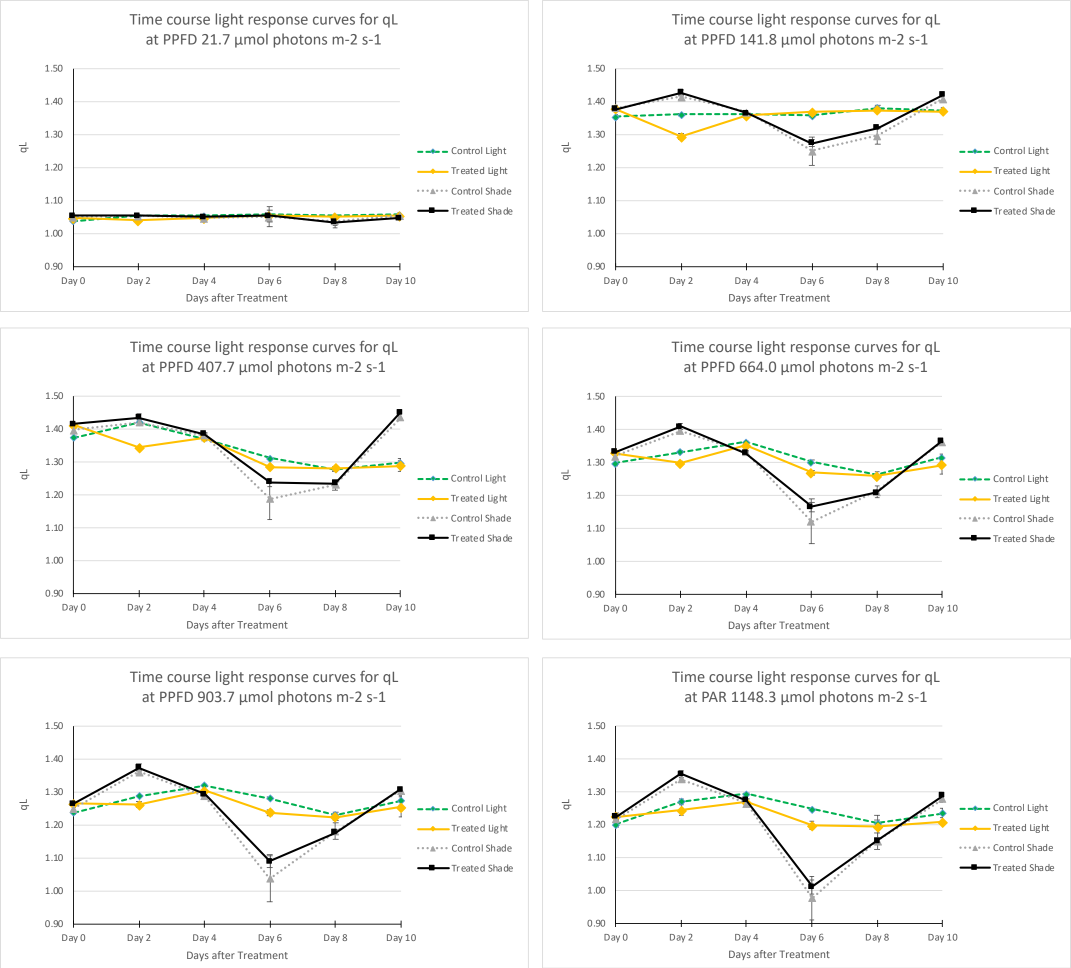


Fig 42. Time course response for qL at each PPDF over the period of the experiment (days after treatment) of turf type *Lolium perenne* (L.) to treatments with 100mg L^{-1} 5-Aminolevulinic acid, applied to plants grown in 100% Daylight (Light) and 50% Daylight (Shade). The chlorophyll fluorescence parameter F_v/F_m was measured on days 0, 2, 4, 6, 8 and 10 after treatments. PPFD = Photosynthetically active Photon Flux Density. Bars show Standard Error of the Mean.

The time course response curves for *qL* are similar to those for ØPSII and F_v/F_m, i.e. a marked decline in value of plants grown in Shade from days 4 to 6 after treatments followed a steep recovery, and ending up higher than plants grown in Light.

iv. *Non-Photochemical Quenching (NVQ)*

There were no significant differences in NPQ due to 5-ALA treatments over the 10-day period of the experiment (Fig 39). Significant differences were observed between plants grown in 100% daylight (Light) and 50% daylight (Shade):

- Day 2 at µmol photons m^{-2} s^{-1} 141.8 (P=0.001), 407.7 (P<0.001), and 664.09 (P<0.022)
- Day 4 at µmol photons m^{-2} s^{-1} 407.7 (P<0.001), 664.0 (P<0.001), 903.7 (P<0.001), and 1148.3 (P<0.001)
- Day 6 at µmol photons m^{-2} s^{-1} 407.7 (P<0.001), and 664.0 (P=0.009)
- Day 8 at photons m^{-2} s^{-1} 21.71 (P=0.022), 141.8 (P=0.014), 407.7 (P<0.001), 664.0 (P<0.001), 903.7 (P=0.002), and 1148.3 (P=0.007)
- Day 10 at µmol photons m^{-2} s^{-1} 141.8 (P<0.001), 407.7 (P<0.001), 664.09 (P<0.001), 903.7 (P=0.003), and 1148.3 (P=0.004)

Gu et al. (2019) state that NPQ initially increases to protect the photosynthetic apparatus, but then decreases when the stress becomes too severe. In Fig 39. such steep increases can be seen in Light grown plants on Days 4, 6 8 and 10, and in Shade grown plants on Days 2 and 8. Figure 43 presents the Time Response Curve at each PPDF used for the fluorescence for each of the days after treatment.

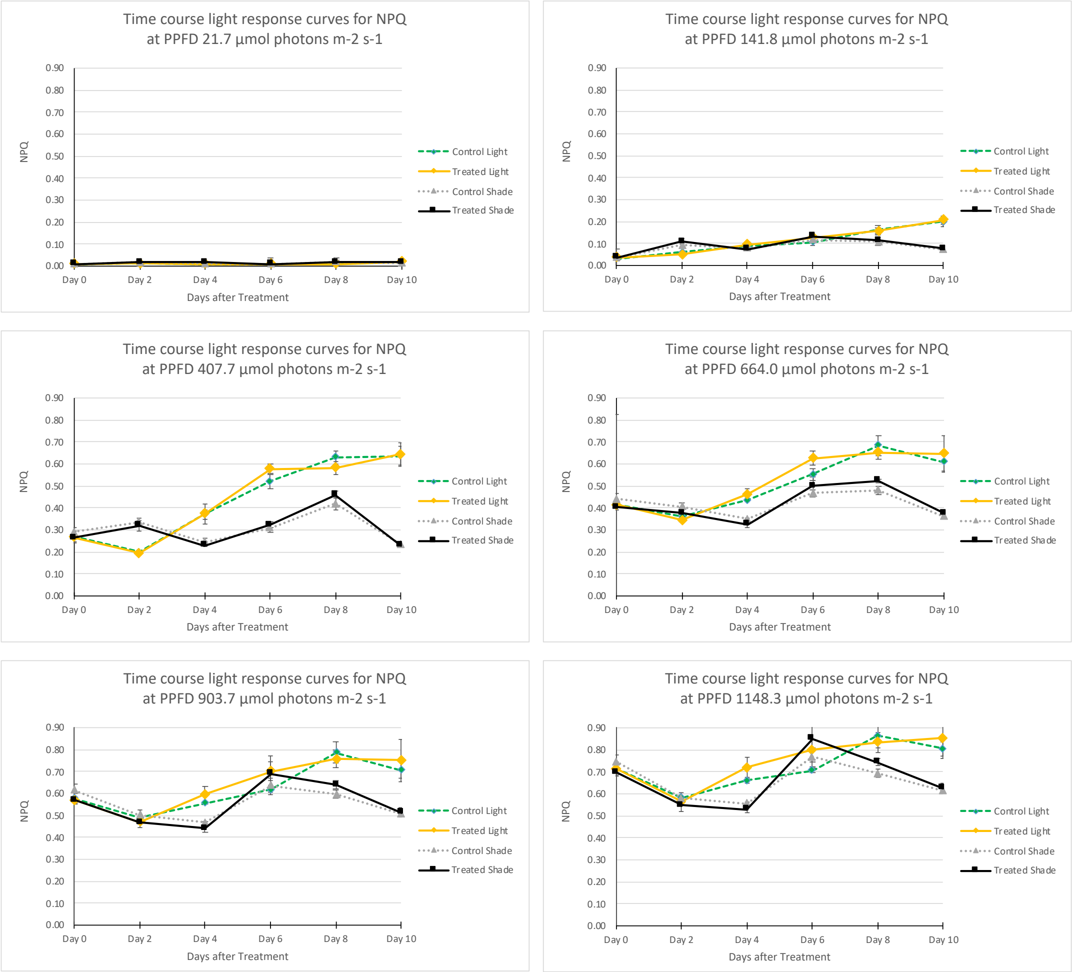


Fig 43. Time course response for NPQ at each PPDF over the period of the experiment (days after treatment) of turf type *Lolium perenne* (L.) to treatments with 100mg L^{-1} 5-Aminolevulinic acid, applied to plants grown in 100% Daylight (Light) and 50% Daylight (Shade). The chlorophyll fluorescence parameter F_v/F_m was measured on days 0, 2, 4, 6, 8 and 10 after treatments. PPFD = Photosynthetically active Photon Flux Density. Bars show Standard Error of the Mean.

The NPQ Time Course Response charts show the mirror results to those for ØPSII, F_v/F_m, and qL. As plant stress increases, as shown by increasing NPQ values, so a fall occurs in ØPSII operating efficiency, the fraction of open PSII reaction centres (qL) and the maximum quantum efficiency of PSII photochemistry (F_v/F_m). The increase in dissipated energy shown by increased NPQ values show the plants are protecting the PSII reaction centres and the shade provided protection from the excess light intensity and heat Wan *et al* (2020).

CHAPTER FOUR

DISCUSSION and CONCLUSION

4.1 Discussion

A discussion of the results of the two experiments cannot be held without taking into account the temperatures and solar irradiation experienced during the period of the experiments. Solar irradiation and temperatures inside the glasshouse between the hours of 7.00am and 7.00pm during the experiments were rarely at the optimum for healthy growth of *L. perenne* L. (Figs 44 and 45 and Appendix 1).

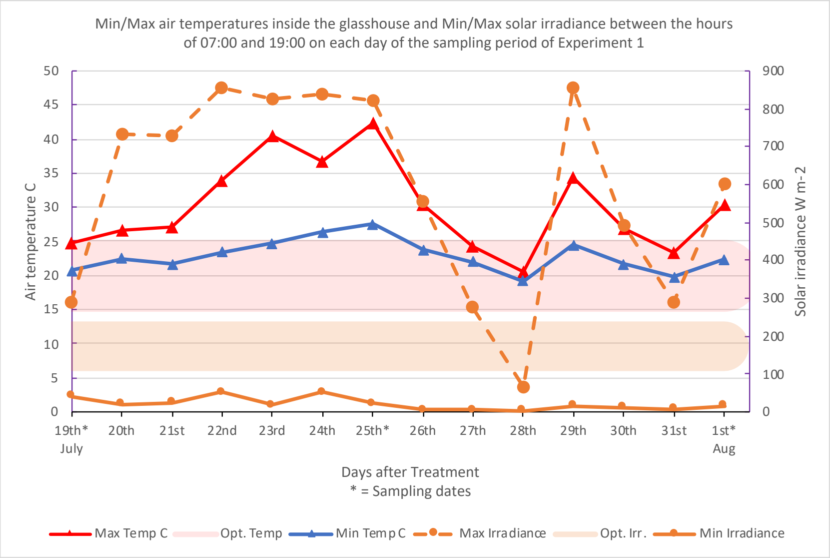


Fig 44. Maximum and minimum measurements of air temperatures (^{0}C) inside the glasshouse and solar irradiance (W m^{-2}) entering he glasshouse on each of the days during sampling of Experiment 1 - turfgrass *Lolium perenne* L. treated with 100mg L^{-1} 5-aminlvulinic acid. Opt. Temp.= optimum temperature range for turfgrass *Lolium perenne* L. range of 15-25^{0}C from Hunter *et al* (2009). Opt. Irr. = optimum solar irradiance for *Lolium perenne* L. (Dudeck and Peacock, 1992). Data from Sutton Bonington weather station.

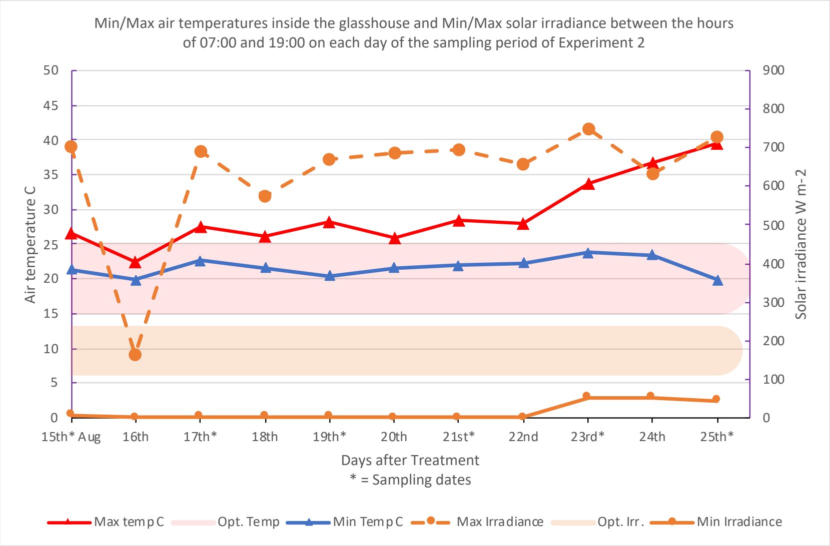


Fig 45. Maximum and minimum measurements of air temperatures (^{0}C) inside the glasshouse and solar irradiance (W m^{-2}) entering the glasshouse during the period of sampling of Experiment 2 - turfgrass *Lolium perenne* L. treated with 100mg L^{-1} 5-aminlvulinic acid. Opt. Temp.= optimum temperature range for turfgrass *Lolium perenne* L. range of 15-25^{0}C from Hunter *et al* (2009). Opt. Irr. = optimum solar irradiance for *Lolium perenne* L. (Dudeck &Peacock, 1992). Data from Sutton Bonington weather station.

The optimum temperatures for shoot growth in cool-season grasses are in the region of 15 to 25^{0}C and, to maintain an acceptable turfgrass surface, perennial ryegrass (*L. perenne* sp.) requires solar irradiance levels between 116 and 233 W m^{-2} (Dudeck and Peacock, 1992), or 11.1 and 20 mol m^{-2} day^{-1} (Steinke and Ervin, 2013). Heat and high irradiance stresses in turfgrass species negatively affects the plant's photosynthesis, respiration, transpiration and water and nutrient uptake (Fry and Huang 2004).

Figs 46 and 47 show samples grown in Shade and Light, respectively, 14 days after treatment, which show the effects of heat and treatments. Treated plants grown in Light show the higher chlorophyll (NDVI) levels. Treated and Non-treated samples grown in Shade show unacceptable condition for a high quality playing surface.

Fig 46. Turfgrass (*L. perenne* L.) grown in 50% Daylight. 14 days after treatment with 100mg L^{-1} 5-ALA. Left- Treated, right- Non-Treated

Fig 47. Turfgrass (*L. perenne* L.) grown in 100% Daylight 14 days after treatment with 100mg L^{-1} 5-ALA. Left- Non-treated, right- Treated

The purpose of this study was to examine if foliar applications of 100mg L^{-1} 5-ALA improved plant health in a low light environment. It is the author's opinion that the experiments were measuring the effects of exogenous applications on turfgrass plants of 100mg L^{-1} 5-ALA on high temperature and light stress in addition to the effects of plants grown in 100% and 50% daylight.

Improving the quality of turfgrass plants grown in sports stadia is crucial to reducing the costs of supplementary lighting that is necessary to maintain high quality natural grass playing surfaces. The high cost of supplementary lighting means that this method is not accessible to many sports clubs, e.g. lower league football clubs, and the pitches suffer in quality from December to end of March due to low light conditions. Low-cost treatments using 5-ALA could be the way to reduce the costs of supplementary lighting and raising the quality of playing surfaces.

In Experiment One NDVI values in plants grown in Shade were significantly higher than non-treated in Shade on day 7 (+19.76%) and on day 14 (+26.69%) after treatment. Treated plants in Light had lower NDVI values than non-treated on days 0 (four hours after treatment) and 7, and higher on day 14 (not significant). Sun *et al* (2009) found an increase in antioxidant enzymes SOD, POD and APX in watermelon in shade after treatment with 100mg L^{-1} 5-ALA and proposed the treatment led to an increase in the electron transfer rate, which alleviated photosynthetic inhibition under low light conditions. Akram and Ashraf (2013) found evidence that 5-ALA, in low light, can speed up the chlorophyll molecule synthesis, and that large numbers of new chlorophyll molecules might be able to capture more available light and attain optimum photosynthetic capacity. The explanations from Sun *et al* (2009) and Akram and Ashraf (2013) were also concluded by other authors, e.g. Hotta *et al* (1998), Korkmax (2012), Dabrowshi *et al* (2015), Wu *et al* (2018). The results from this study would indicate that in low light conditions applications of 100mg L^{-1} of 5-ALA increases chlorophyll content but may suppress chlorophyll production in normal daylight (see Fig 47).

As with the results for NDVI values, % Dry Weight was also significantly higher on day 7 after treatment than in non-treated plants in plants grown in Shade. In Experiment One, days 0-7 after treatment was a continuous period when the highest measurements of solar irradiation and heat were recorded. This suggests that 5-ALA increases chlorophyll and mitigates heat stress in *L. perenne* grown in 50% daylight.

The effects on nutrient content (mg kg^{-1}) of applications of 100mg L^{-1} 5-ALA was inconclusive. It has been reported that applications of 5-ALA based liquid fertilisers enhance the nutrient content of plants, e.g. (Tilly-Mándy *et al*, 2010; Korkmaz, 2012; Song *et al*, 2017; Anwar *et al*, 2018: Wu *et al.* 2019), and the results from this study align with those found by Xu *et al* (2010) in that 5-ALA increases some nutrients in treated plants. This study, however, did not find an increase in S as reported by Wu *et al.* (2019) who hypothesized that an increase in the biosynthesis of siroheme through applications of 5-ALA is linked to an increase in the uptake of nitrogen and sulphur. Nor did this study find increases of P, K and Ca concentrations as reported by Xu *et al* (2010). Korkmaz (2012) proposed that in light ALA is synthesised in optimal amounts by the plant and may explain the lack of responses. It must also be born in mind that in this study fertiliser applications mirrored those as applied to English Premiership football pitches and the turfgrass plants suffered no deficits of any mineral nutrient nor drought stress.

Fig 45 shows that the maximum air temperatures and solar irradiance on each of the Experiment Two sampling days were consistently above optimum levels for healthy turfgrass growth. The deleterious effects of high temperatures and solar irradiance on PSII photosynthesis is shown on Day 6 after treatment in the sharp decrease in values for ØPSII, F_v/F_m, and qL followed by rises in values, particularly in plants grown in Shade. As a fall occurs in the fraction of open PSII reaction centres (qL) and the maximum quantum efficiency of PSII photochemistry (F_v/F_m), so ØPSII operating efficiency falls. This results in an increase in dissipated energy shown by increased NPQ values, showing that the plants are protecting the PSII reaction centres by shedding excess energy via heat, but shade provided protection from the excess light intensity. The occurrence of reduced F_v/F_m in sunlight, with an accompanying photoinhibition, agrees with Dabrowski *et al* (2015) that the plant is spending energy in mitigating the effects of high light levels, but recovery in the Light Harvesting Complexes occurs when conditions become more favourable.

Turfgrass plants adapt to shade 4-7 days from the onset of shade stress but at the expense of all plant processes (Gardener and Goss, 2013). The sharp dip in ØPSII, F_v/F_m, and qL in Shade could be the plants suffering from the effects of reduced light on photosynthesis followed by a recovery from Days 6 to 10 in ØPSII as the processes adapt to the lower light levels, with values in Shade exceeding plants grown in Light. This is could be related to the increase in high temperatures and associated stress on ØPSII in Light. Turfgrass samples grown in shade were able to photosynthesise more than grass exposed to full sunlight during periods of high intensity sunlight, i.e. there was the possibility of photoinhibition occurring in plants grown in Light. CO_2 assimilation in turfgrass plants grown in low light, however, is severely restricted when subject to high temperature stress. As temperatures increase the solubility of CO_2 decreases, which reduces CO_2 concentration and decreases RuBP carboxylase activity, the enzyme that functions as the acceptor molecule and initial reductant for CO_2 (Hull, 1992). Whilst photosynthesis rates decrease in response to high temperature, respiration and photorespiration rates continue to increase until carbohydrate reserves are depleted (DaCosta and Huang, 2013).

Applications of 100mg L^{-1} 5-aminlevulinic acid (5-ALA) had little effect during this experiment with most significant differences were attributed if the turfgrass plants were grown in Light or Shade, not to 5-ALA applications. Significant differences due to 5-ALA occurred in *qL* in Light grown plants on Days 0 (four hours) and 2 after treatments but showed decreases on Day 2 (Figs. 39 and 42). The only significant effect on ØPSII from 5-ALA was also seen on Day 0 after treatment with an increase value at PPFD 1148.3 in Treated Shade plants, indicating that 5-ALA had a short-term beneficial effect on plants grown in Shade. 5-ALA treatments had a significant effect on F_v/F_m only on Days 0 and 2 in plants grown in 100% daylight, indicating that 5-ALA treatments could benefit grass grown in shaded areas for a short period of time after application.

4.2 Conclusion

The breadth of data from the two experiments supports the conclusion that exogenous treatments of 100mg L^{-1} 5-Aminolvulinic acid (5-ALA) can have some effects on the growth and development of turfgrass species *L. perenne*. Some differences were observed from applications of 5-ALA but the grass struggled to deal with excessive heat and light, particularly in the early stages of growth. Lower concentrations of 5-ALA might be more beneficial and running the experiment in a more controlled environment, where light and heat can be better regulated, could demonstrate beneficial effects of 5-ALA on turfgrass species. Other effects of 5-ALA to investigate, and which would be of benefit to turfgrass managers, especially to facilities with lower spending budgets than Premier League club:

- which Reactive Oxygen Species and antioxidants are involved?
- the carbon energy partitioning between roots and leaves
- root:leaf ratio by dry weight
- in low nutrient conditions
- on the effects of drought
- pre-treating the seed
- dose rates and timing
- CO_2 uptake and exchange

This study highlighted the importance of a larger number of samples required for more accurate analysis. The tubs enabled a full rootzone profile to be used that mimicked modern construction profiles with accompanying water drainage and fertiliser applications, but carrying out the experiments on outside plots during the summer would provide a larger area for sampling and reduce errors in sampling and statistical analysis.

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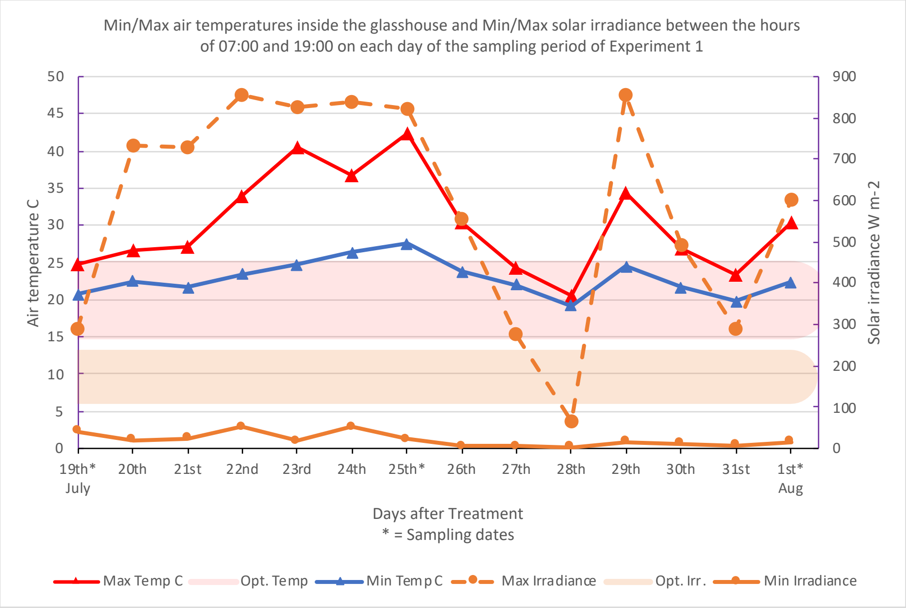
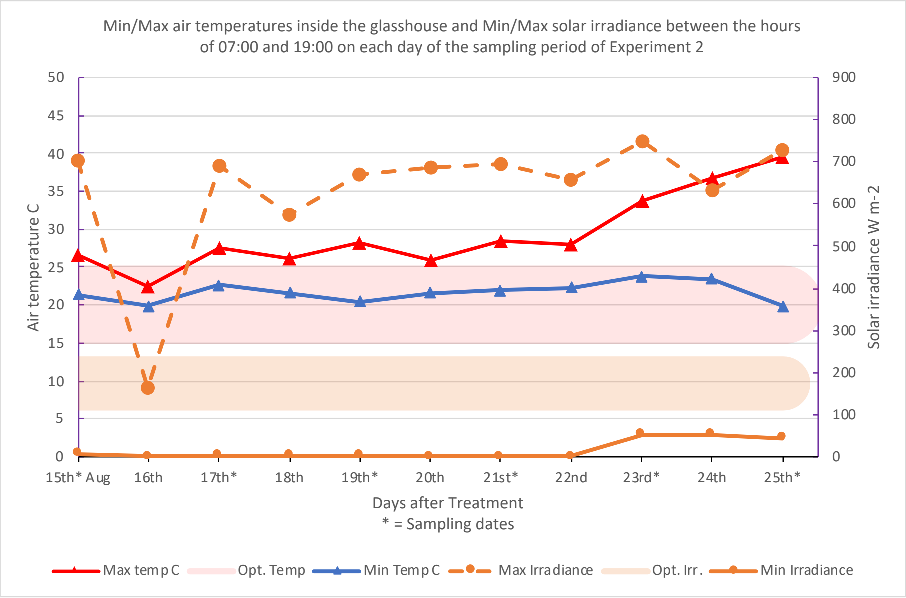
APPENDIX I

Air Temperatures and Solar Irradiance measurements

Measurements of air temperatures (^{0}C) and solar irradiance (W m^{-2}) on during the period of sampling of turfgrass *L. perenne* L. after treatments of 100mg L^{-1} 5-aminlvulinic acid. Opt. Temp.= optimum temperature range for turfgrass *L. perenne* range of 15-25^{0}C from Hunter *et al* (2009). Opt. Irr. = optimum solar irradiance for *L. perenne* (Cockerham *et al* 2002), converted from mol m^{-2}day^{-1} to W m^{-2}, range of 11-20 m^{-2}day^{-1} to W m^{-2}.



Min/Max air temperatures inside the glasshouse and Min/Max solar irradiance between the hours of 07:00 and 19:00 on each day of the sampling period of Experiment 1



Min/Max air temperatures inside the glasshouse and Min/Max solar irradiance between the hours of 07:00 and 19:00 on each day of the sampling period of Experiment 2

Air Temperature (^{o}C) and Solar Irradiation (W/m^2) for Experiment One
Green shaded data are sampling days

Sample No.	Date	Time (24hr)	Air Temp ^{o}C	Irradiance W/m2	Irr. (µmol m-2 s-1)	Opt. Temp. C	Opt. Irr. (W/m2)
1 (4 hrs after treatment)	**19th July**	7	22.36	150.005	690.02	20	108
		8	21.57	250.3283	1151.51	20	108
		9	21.44	130.2433	599.12	20	108
		10	22.10	123.7283	569.15	20	108
		11	23.71	122.3533	562.83	20	108
		12	22.20	290.5183	1336.38	20	108
		13	23.86	120.9133	556.20	20	108
		14	24.74	161.7283	743.95	20	108
		15	22.76	175.82	808.77	20	108
		16	21.92	87.775	403.77	20	108
		17	21.28	42.18	194.03	20	108
		18	22.55	21.7683	100.13	20	108
		19	22.76	18.1333	83.41	20	108
	20th	7	24.10	204.6	941.16	20	108
		8	24.26	212.75	978.65	20	108
		9	23.71	334.0967	1536.84	20	108
		10	25.66	341.6017	1571.37	20	108
		11	26.66	438.3583	2016.45	20	108
		12	25.66	731.1967	3363.50	20	108
		13	25.06	281.2417	1293.71	20	108
		14	26.91	280.7833	1291.60	20	108
		15	24.69	543.45	2499.87	20	108
		16	24.66	359.9583	1655.81	20	108
		17	22.53	360.5283	1658.43	20	108
		18	21.69	135.3483	622.60	20	108
		19	23.61	19.7083	90.66	20	108
	21st	7	25.83	229.565	1056.00	20	108
		8	27.14	423.1483	1946.48	20	108
		9	26.10	586.365	2697.28	20	108
		10	27.22	525.585	2417.69	20	108
		11	27.02	730.3067	3359.41	20	108
		12	25.83	631.2967	2903.96	20	108
		13	25.63	363.4533	1671.89	20	108
		14	26.28	298.1133	1371.32	20	108
		15	25.28	307.1833	1413.04	20	108
		16	24.04	240.1917	1104.88	20	108
		17	23.46	170.815	785.75	20	108
		18	23.44	81.0217	372.70	20	108
		19	23.68	24	110.40	20	108
	22nd	7	24.80	128.0033	588.82	20	108
		8	29.36	163.625	752.68	20	108
		9	31.69	593.4933	2730.07	20	108
		10	31.83	766.2533	3524.77	20	108
		11	32.05	854.9533	3932.79	20	108
		12	33.84	812.725	3738.54	20	108
		13	33.58	799.7533	3678.87	20	108
		14	33.79	738.6433	3397.76	20	108
		15	33.22	629.075	2893.75	20	108
		16	30.96	560.205	2576.94	20	108
		17	27.88	371.8333	1710.43	20	108
		18	27.21	173.895	799.92	20	108
		19	24.72	52.8	242.88	20	108
	23rd	7	26.47	254.46	1170.52	20	108
		8	32.60	279.71	1286.67	20	108
		9	34.54	531.2533	2443.77	20	108
		10	35.81	770.69	3545.17	20	108
		11	36.93	806.9067	3711.77	20	108
		12	38.95	826.0467	3799.81	20	108
		13	40.12	785.09	3611.41	20	108

		14	40.39	707.4467	3254.25	20	108
		15	37.64	600.1983	2760.91	20	108
		16	33.95	456.1	2098.06	20	108
		17	31.06	261.755	1204.07	20	108
		18	26.39	96.86	445.56	20	108
		19	30.37	19	87.40	20	108
	24th	7	28.64	395.0317	1817.15	20	108
		8	28.77	420.605	1934.78	20	108
		9	31.84	460.8617	2119.96	20	108
		10	32.81	644.705	2965.64	20	108
		11	35.55	733.8267	3375.60	20	108
		12	36.65	839.1067	3859.89	20	108
		13	35.20	792.515	3645.57	20	108
		14	33.50	698.7433	3214.22	20	108
		15	30.94	484.7483	2229.84	20	108
		16	29.60	320.7333	1475.37	20	108
		17	28.11	167.6533	771.21	20	108
		18	27.50	89.3683	411.09	20	108
		19	31.36	52.9017	243.35	20	108
2	**25th**	7	32.41	394.365	1814.08	20	108
		8	35.26	544.295	2503.76	20	108
		9	37.72	665.25	3060.15	20	108
		10	38.28	763.32	3511.27	20	108
		11	40.18	821.1017	3777.07	20	108
		12	42.43	737.3	3391.58	20	108
		13	38.76	660.9267	3040.26	20	108
		14	39.71	363.01	1669.85	20	108
		15	39.01	394.9083	1816.58	20	108
		16	36.47	365.855	1682.93	20	108
		17	33.03	125.365	576.68	20	108
		18	23.81	81.2733	373.86	20	108
		19	25.13	22.1267	101.78	20	108
	26th	7	26.67	130.125	598.58	20	108
		8	29.10	219.165	1008.16	20	108
		9	27.99	330.4633	1520.13	20	108
		10	29.83	332.77	1530.74	20	108
		11	29.04	301.395	1386.42	20	108
		12	30.43	338.7517	1558.26	20	108
		13	27.22	557.2533	2563.37	20	108
		14	26.99	150.3617	691.66	20	108
		15	28.83	100.985	464.53	20	108
		16	26.59	211.9083	974.78	20	108
		17	25.13	130.325	599.50	20	108
		18	22.09	26.81	123.33	20	108
		19	22.48	6.3583	29.25	20	108
	27th	7	23.93	88.7033	408.04	20	108
		8	22.35	129.8017	597.09	20	108
		9	22.38	73.435	337.80	20	108
		10	22.00	74.905	344.56	20	108
		11	22.09	93.5133	430.16	20	108
		12	22.18	88.2767	406.07	20	108
		13	24.17	104.5	480.70	20	108
		14	23.41	214.755	987.87	20	108
		15	22.55	274.9717	1264.87	20	108
		16	21.74	141.78	652.19	20	108
		17	20.78	83.4817	384.02	20	108
		18	20.35	30.615	140.83	20	108
		19	20.59	5.8667	26.99	20	108
	28th	7	19.54	24.34	111.96	20	108
		8	20.54	22.9567	105.60	20	108
		9	19.58	51.6183	237.44	20	108
		10	19.41	56.2667	258.83	20	108
		11	19.91	48.39	222.59	20	108

		12	19.75	61.945	284.95	20	108
		13	19.69	64.9317	298.69	20	108
		14	19.21	71.3683	328.29	20	108
		15	20.33	55.8217	256.78	20	108
		16	20.07	33.345	153.39	20	108
		17	19.33	22.74	104.60	20	108
		18	24.43	13.365	61.48	20	108
		19	26.56	2.7533	12.67	20	108
	29th	7	26.43	414.23	1905.46	20	108
		8	26.52	556.9633	2562.03	20	108
		9	30.05	638.3967	2936.62	20	108
		10	29.98	805.4433	3705.04	20	108
		11	30.54	842.9167	3877.42	20	108
		12	32.45	856.19	3938.47	20	108
		13	32.66	588.7583	2708.29	20	108
		14	34.47	630.07	2898.32	20	108
		15	32.31	569.3383	2618.96	20	108
		16	32.07	386.2833	1776.90	20	108
		17	25.93	310.615	1428.83	20	108
		18	26.57	72.0017	331.21	20	108
		19	24.38	16.9233	77.85	20	108
	30th	7	26.75	385.73	1774.36	20	108
		8	25.27	501.635	2307.52	20	108
		9	26.81	392.6267	1806.08	20	108
		10	23.96	493.6567	2270.82	20	108
		11	25.52	173.4067	797.67	20	108
		12	23.57	198.2883	912.13	20	108
		13	23.59	263.9867	1214.34	20	108
		14	21.93	391.4067	1800.47	20	108
		15	21.66	118.73	546.16	20	108
		16	21.99	65.0367	299.17	20	108
		17	21.61	52.8667	243.19	20	108
		18	19.81	50.09	230.41	20	108
		19	20.10	12.2417	56.31	20	108
	31st	7	20.06	50.43	231.98	20	108
		8	20.78	60.62	278.85	20	108
		9	22.86	92.4917	425.46	20	108
		10	23.18	214.07	984.72	20	108
		11	22.55	202.5933	931.93	20	108
		12	22.82	305.095	1403.44	20	108
		13	23.44	180.12	828.55	20	108
		14	23.24	289.0667	1329.71	20	108
		15	22.52	186.1733	856.40	20	108
		16	21.93	214.235	985.48	20	108
		17	21.50	88.6783	407.92	20	108
		18	22.40	33.8883	155.89	20	108
		19	22.98	7.0167	32.28	20	108
3	**1 st Aug**	7	25.71	154.575	711.05	20	108
		8	26.35	290.1433	1334.66	20	108
		9	30.14	496.6183	2284.44	20	108
		10	29.70	607.6233	2795.07	20	108
		11	30.39	581.8383	2676.46	20	108
		12	29.79	785.405	3612.86	20	108
		13	26.51	600.0617	2760.28	20	108
		14	26.76	267.89	1232.29	20	108
		15	25.42	249.4767	1147.59	20	108
		16	23.49	155.475	715.19	20	108
		17	22.58	105.3733	484.72	20	108
		18	22.36	59.1183	271.94	20	108
		19	21.57	15.0917	69.42	20	108

Air Temperature (^{o}C) and Solar Irradiation (W/m^2) for Experiment Two
Green shaded data are sampling days

Sample No.	Date	Time (24hr)	Air Temp C	Irradiance W/m2	Irr. (µmol m-2 s-1)	Opt. Temp. C	Opt. Irr. (Wm2)
1 (4 hrs after tretament)	15th Aug	7	23.45	206.3333	949.13	20	175
		8	21.38	194.7983	896.07	20	175
		9	21.97	236.9267	1089.86	20	175
		10	22.32	423.2767	1947.07	20	175
		11	24.87	569.89	2621.49	20	175
		12	26.27	636.1133	2926.12	20	175
		13	24.95	704.275	3239.67	20	175
		14	26.52	671.8633	3090.57	20	175
		15	24.90	480.4633	2210.13	20	175
		16	23.22	229.61	1056.21	20	175
		17	23.78	164.3567	756.04	20	175
		18	22.85	90.55	416.53	20	175
		19	21.47	6.6283	30.49	20	175
	16th	7	19.92	106.1167	488.14	20	175
		8	21.02	110.3367	507.55	20	175
		9	21.41	133.1817	612.64	20	175
		10	22.37	165.7783	762.58	20	175
		11	21.91	150.4167	691.92	20	175
		12	22.84	123.7767	569.37	20	175
		13	21.48	104.2067	479.35	20	175
		14	21.62	132.2283	608.25	20	175
		15	21.82	85.5017	393.31	20	175
		16	21.14	68.5717	315.43	20	175
		17	20.57	30.145	138.67	20	175
		18	19.57	7.145	32.87	20	175
		19	18.96	1.6183	7.44	20	175
2	17th	7	23.69	215.79	992.63	20	175
		8	22.59	461.6967	2123.80	20	175
		9	25.87	504.9983	2322.99	20	175
		10	27.24	571.7233	2629.93	20	175
		11	25.82	691.8317	3182.43	20	175
		12	25.25	647.7183	2979.50	20	175
		13	26.63	658.07	3027.12	20	175
		14	27.45	522.6267	2404.08	20	175
		15	27.32	466.3817	2145.36	20	175
		16	25.89	279.4967	1285.68	20	175
		17	24.94	225.1483	1035.68	20	175
		18	24.65	95.9783	441.50	20	175
		19	22.65	4.2083	19.36	20	175
	18th	7	23.06	276.285	1270.91	20	175
		8	23.25	408.0867	1877.20	20	175
		9	26.27	416.95	1917.97	20	175
		10	23.74	475.6267	2187.88	20	175
		11	25.97	549.4667	2527.55	20	175
		12	26.36	572.655	2634.21	20	175
		13	26.14	504.915	2322.61	20	175
		14	24.52	213.115	980.33	20	175
		15	24.11	228.575	1051.45	20	175
		16	25.38	358.725	1650.14	20	175
		17	23.62	111.255	511.77	20	175
		18	22.24	65.8717	303.01	20	175
		19	21.59	2.7567	12.68	20	175
3	19th	7	20.45	243.5883	1120.51	20	175
		8	25.45	305.1583	1403.73	20	175
		9	23.24	456.7583	2101.09	20	175
		10	25.07	507.165	2332.96	20	175
		11	23.57	668.5483	3075.32	20	175
		12	28.31	308.1567	1417.52	20	175
		13	25.36	547.7817	2519.80	20	175

		14	23.56	475.475	2187.19	20	175
		15	26.26	400.4117	1841.89	20	175
		16	25.46	386.5317	1778.05	20	175
		17	24.96	216.8683	997.59	20	175
		18	23.42	72.68	334.33	20	175
		19	21.37	2.715	12.49	20	175
	20th	7	21.78	314.5183	1446.78	20	175
		8	23.62	398.5883	1833.51	20	175
		9	23.82	380.4483	1750.06	20	175
		10	23.88	367.39	1689.99	20	175
		11	24.86	452.1083	2079.70	20	175
		12	25.46	514.0333	2364.55	20	175
		13	26.02	684.4033	3148.26	20	175
		14	24.24	550.8233	2533.79	20	175
		15	23.85	462.6567	2128.22	20	175
		16	26.58	288.0067	1324.83	20	175
		17	23.96	163.9433	754.14	20	175
		18	22.23	35.4267	162.96	20	175
		19	21.62	2.06	9.48	20	175
4	**21st**	7	22.08	221.3883	1018.39	20	175
		8	22.48	310.09	1426.41	20	175
		9	24.43	543.9683	2502.25	20	175
		10	27.29	532.8667	2451.19	20	175
		11	28.36	547.2917	2517.54	20	175
		12	26.79	693.1233	3188.37	20	175
		13	27.59	429.4083	1975.28	20	175
		14	26.80	358.8317	1650.63	20	175
		15	24.91	396.7567	1825.08	20	175
		16	26.72	286.0033	1315.62	20	175
		17	24.95	208.3033	958.20	20	175
		18	23.42	32.8033	150.90	20	175
		19	21.92	1.3883	6.39	20	175
	22nd	7	22.35	254.935	1172.70	20	175
		8	23.38	419.3383	1928.96	20	175
		9	26.06	495.4917	2279.26	20	175
		10	25.86	416.835	1917.44	20	175
		11	24.69	363.0867	1670.20	20	175
		12	25.11	634.5217	2918.80	20	175
		13	26.61	658.995	3031.38	20	175
		14	27.89	319.6017	1470.17	20	175
		15	25.87	261.3067	1202.01	20	175
		16	24.07	77.71	357.47	20	175
		17	23.83	117.4933	540.47	20	175
		18	23.14	44.1867	203.26	20	175
		19	22.33	0.9317	4.29	20	175
5	**23rd**	7	23.76	316.0533	1453.85	20	175
		8	25.66	488.1817	2245.64	20	175
		9	28.12	483.0783	2222.16	20	175
		10	29.14	637.235	2931.28	20	175
		11	31.89	606.3633	2789.27	20	175
		12	30.28	748.7683	3444.33	20	175
		13	31.50	696.1167	3202.14	20	175
		14	33.29	576.4017	2651.45	20	175
		15	32.80	498.6267	2293.68	20	175
		16	33.69	349.315	1606.85	20	175
		17	33.40	189.0717	869.73	20	175
		18	29.86	53.4117	245.69	20	175
		19	26.31	-1.74	-8.00	20	175
	24th	7	23.44	299.3217	1376.88	20	175
		8	27.04	473.6467	2178.77	20	175
		9	29.61	624.3	2871.78	20	175
		10	31.64	724.1133	3330.92	20	175
		11	34.43	696.115	3202.13	20	175

		12	33.09	474.8667	2184.39	20	175
		13	32.68	634.2233	2917.43	20	175
		14	35.67	616.235	2834.68	20	175
		15	36.72	493.1517	2268.50	20	175
		16	36.16	252.76	1162.70	20	175
		17	32.71	157.9683	726.65	20	175
		18	31.86	53.345	245.39	20	175
		19	26.98	-2.7817	-12.80	20	175
6	**25th**	7	19.85	275.1117	1265.51	20	175
		8	27.20	436.6417	2008.55	20	175
		9	30.20	570.555	2624.55	20	175
		10	32.99	667.5617	3070.78	20	175
		11	34.90	725.8017	3338.69	20	175
		12	34.99	727.6367	3347.13	20	175
		13	36.03	683.29	3143.13	20	175
		14	39.02	590.8783	2718.04	20	175
		15	39.58	471.0367	2166.77	20	175
		16	38.42	323.4333	1487.79	20	175
		17	37.52	168.3667	774.49	20	175
		18	34.65	44.1367	203.03	20	175
		19	29.40	-1.2867	-5.92	20	175

APPENDIX II

Results for Dry Weight (%)

Leaf Dry Weights (%) of clippings mown at 30mm

Means with same letters within each sample day after treatment are not significantly different at P≤ 0.05 according to Duncan's Multiple Range

Date	Treatment	Mean		St Error
Day 0	Control Light	18.62%ab	±	0.003
	Treated Light	20.12%b	±	0.010
	Control Shade	17.19%a	±	0.010
	Treated Shade	17.43%a	±	0.002
Day 7	Control Light	14.34%ab	±	0.009
	Treated Light	15.94%b	±	0.006
	Control Shade	11.02%a	±	0.001
	Treated Shade	14.20%ab	±	0.023
Day 14	Control Light	13.28%b	±	0.025
	Treated Light	17.79%b	±	0.022
	Control Shade	7.88%a	±	0.023
	Treated Shade	9.62%a	±	0.017

ANOVA for Chlorophyll (NDVI)

Yellow highlight indicates significant differences at P≤ 0.05

Day 0

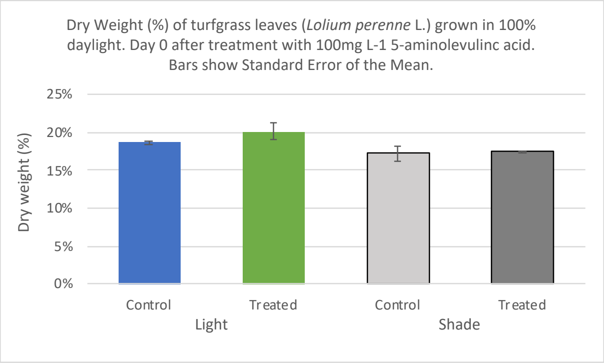
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0002273	0.0002273	1.31	0.285
Light	1	0.0012756	0.0012756	7.36	0.027
ALA.Light	1	0.0001192	0.0001192	0.69	0.431
Residual	8	0.0013859	0.0001732		
Total	11	0.0030080			

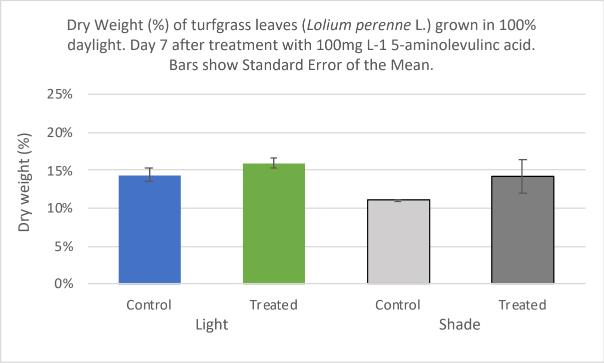
Day 7

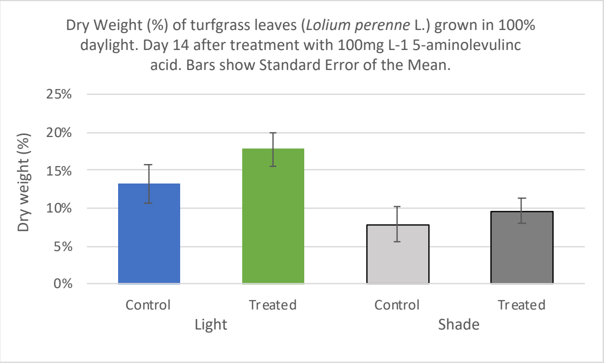
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0017089	0.0017089	3.57	0.096
Light	1	0.0019235	0.0019235	4.02	0.080
ALA.Light	1	0.0001856	0.0001856	0.39	0.551
Residual	8	0.0038300	0.0004788		
Total	11	0.0076480			

Day 14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.002925	0.002925	2.04	0.191
Light	1	0.013796	0.013796	9.63	0.015
ALA.Light	1	0.000573	0.000573	0.40	0.545
Residual	8	0.011456	0.001432		
Total	11	0.028750			


Dry Weight (%) of turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight. Day 0 after treatment with 100mg L-1 5-aminolevulinc acid. Bars show Standard Error of the Mean.


Dry Weight (%) of turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight. Day 7 after treatment with 100mg L-1 5-aminolevulinc acid. Bars show Standard Error of the Mean.


Dry Weight (%) of turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight. Day 14 after treatment with 100mg L-1 5-aminolevulinc acid. Bars show Standard Error of the Mean.

APPENDIX III

Results for chlorophyll content (NDVI)

Chlorophyll values (NVDI)

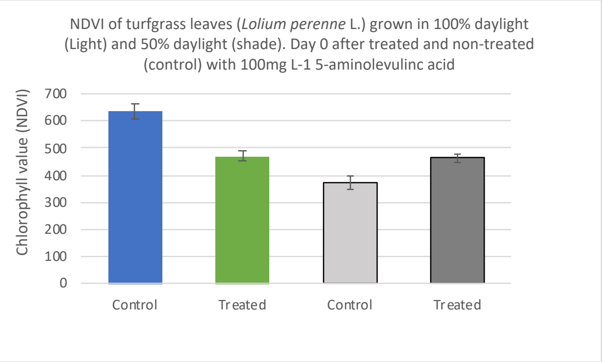
Means with same letters within each row or column of each data set are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

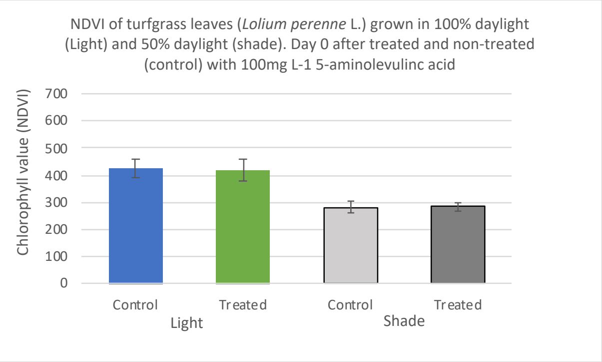
Date	Treatment	Mean		St Error
Day 0	Control Light	425.22b	±	36.426
	Treated Light	420.33b	±	42.179
	Control Shade	282.11a	±	22.245
	Treated Shade	284.44a	±	15.789
Day 7	Control Light	636.56c	±	27.736
	Treated Light	470.67b	±	18.030
	Control Shade	373.11a	±	22.164
	Treated Shade	465.00b	±	15.861
Day 14	Control Light	364.78bc	±	25.486
	Treated Light	391.33c	±	25.287
	Control Shade	222.22a	±	19.260
	Treated Shade	303.11b	±	19.572

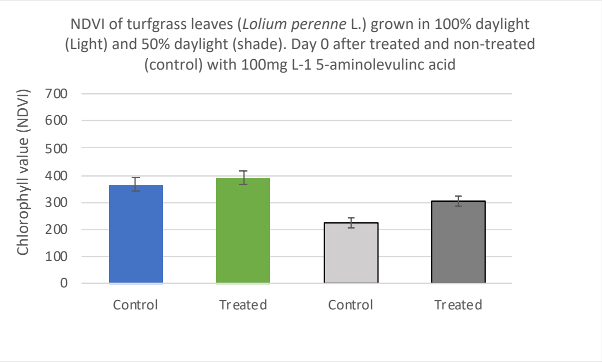
ANOVA for Chlorophyll (NDVI)

Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	15.	15.	0.00	0.967
Light	1	175142.	175142.	20.22	<.001
ALA.Light	1	117.	117.	0.01	0.908
Residual	32	277201.	8663.		
Total	35	452475.			
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	12321.	12321.	2.98	0.094
Light	1	162947.	162947.	39.42	<.001
ALA.Light	1	149511.	149511.	36.17	<.001
Residual	32	132281.	4134.		
Total	35	457060.			
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	25975.	25975.	5.65	0.024
Light	1	119831.	119831.	26.07	<.001
ALA.Light	1	6642.	6642.	1.45	0.238
Residual	32	147094.	4597.		
Total	35	299542.			







Appendix IV - Mean Leaf Nutrient Content (mg kg^{-1})

Effects of treatment with 100mg kg^{-1} 5-Aminolevulinic acid (5-ALA) on nutrient content (mg kg^{-1}) in turfgrass type *L. perenne* L. grown in 100% and 50% daylight. Samples taken 0, 7 and 14 days after treatment. Means with same letters within each column of each nutrient data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test. Treatments: Control (non-treated) in 100% daylight, Treated in 100% daylight, Control (non-treated) in 50% daylight, Treated in 50% daylight.

Day 0 (4 hours) after treatment

Nutrient		**P**	**St. Error**	**K**	**St. Error**	**Ca**	**St. Error**	**Mg**	**St. Error**	**S**	**St. Error**	**Na**	**St. Error**	**B**	**St. Error**
100% Daylight	**Control**	6188a	± 124	48994a	± 1546	3979a	± 156	2679ab	± 46	5872a	± 12	162.12b	± 10.32	6.50ab	± 0.55
(Light)	**Treated**	5940a	± 170	46790a	± 2687	4110a	± 152	2484a	± 48	5657a	± 172	182.50a	± 8.32	7.16b	± 0.48
50% Daylight	**Control**	6346a	± 204	48165a	± 1568	4088a	± 70	2748b	± 10	5732a	± 151	182.96a	± 5.03	5.63ab	± 0.7
(Shade)	**Treated**	6094a	± 234	47560a	± 1111	3946a	± 175	2646ab	± 96	5810a	± 254	164.16b	± 6.08	4.77a	± 0.13
Nutrient		**Fe**	**St. Error**	**Mn**	**St. Error**	**Mo**	**St. Error**	**Cu**	**St. Error**	**Zn**	**St. Error**	**Se**	**St. Error**	**Co**	**St. Error**
100% Daylight	**Control**	201.24ab	± 32.08	179.48a	± 3.01	3.10a	± 0.05	12.73a	± 0.16	51.13b	± 0.39	0.047a	± 0.002	0.112a	± 0.001
(Light)	**Treated**	206.13b	± 24.7	184.51a	± 7.17	3.09a	± 0.12	12.55a	± 0.44	46.81a	± 2.06	0.046a	± 0.002	0.122a	± 0.006
50% Daylight	**Control**	145.50ab	± 7.93	174.04a	± 3.22	3.08a	± 0.16	13.34a	± 0.29	49.66ab	± 0.68	0.048a	± 0.002	0.112a	± 0.00
(Shade)	**Treated**	144.46a	± 10.72	183.58a	± 7.61	2.98a	± 0.25	12.92a	± 0.20	52.08b	± 1.19	0.043a	± 0.002	0.117a	± 0.004

Day 7 after treatment

Nutrient		**P**	**St. Error**	**K**	**St. Error**	**Ca**	**St. Error**	**Mg**	**St. Error**	**S**	**St. Error**	**Na**	**St. Error**	**B**	**St. Error**
100% Daylight	**Control**	5665a	± 246	44378a	± 2230	3635ab	± 91	2626ab	± 28	6162a	± 209	212.97a	± 7.09	7.46a	± 1.1
(Light)	**Treated**	6336a	± 332	44071a	± 1292	3876b	± 60	2538b	± 1	6631a	± 151	241.86a	± 28.55	10.00a	± 0.87
50% Daylight	**Control**	5993a	± 216	41502a	± 1461	3644ab	± 153	2308a	± 31	5975a	± 465	234.56a	± 20.87	7.20 a	± 0.31
(Shade)	**Treated**	6343a	± 128	43590a	± 665	3457a	± 139	2377a	± 112	6385a	± 463	238.36a	± 19.48	8.18a	± 0.76
Nutrient		**Fe**	**St. Error**	**Mn**	**St. Error**	**Mo**	**St. Error**	**Cu**	**St. Error**	**Zn**	**St. Error**	**Se**	**St. Error**	**Co**	**St. Error**
100% Daylight	**Control**	190.69ab	± 24.9	152.73a	± 4.44	3.41a	± 0.13	11.07a	± 0.10	47.49a	± 3.23	0.057a	± 0.005	0.094a	± 0.003
(Light)	**Treated**	280.89b	± 73.82	181.95b	± 2.43	3.84a	± 0.12	11.59a	± 0.04	57.86a	± 5.91	0.066a	± 0.008	0.125b	± 0.010
50% Daylight	**Control**	167.70ab	± 28.41	163.22a	± 1.88	3.67a	± 0.12	11.37a	± 0.57	58.54a	± 13.41	0.062a	± 0.008	0.105ab	± 0.007
(Shade)	**Treated**	135.70a	± 6.76	156.76a	± 4.74	3.51a	± 0.18	11.73a	± 0.43	47.87a	± 2.39	0.057a	± 0.001	0.091a	± 0.007

Day 14 after treatment

Nutrient		**P**	**St. Error**	**K**	**St. Error**	**Ca**	**St. Error**	**Mg**	**St. Error**	**S**	**St. Error**	**Na**	**St. Error**	**B**	**St. Error**
100% Daylight	**Control**	4893a	± 13780	36470a	± 11817	3723a	± 1211	2371a	± 742	5445a	± 1615	190.30a	± 60.88	3.69a	± 0.98
(Light)	**Treated**	4845 a	± 147	33999a	± 210	3172a	± 48	2034a	± 55	4962a	± 47	207.76a	± 14.22	3.16a	± 0.07
50% Daylight	**Control**	5303a	± 289	34854a	± 3836	3812a	± 263	2335a	± 241	4002a	± 244	330.31b	± 54.27	3.79a	± 0.07
(Shade)	**Treated**	5725a	± 511	34262a	± 4262	4324a	± 713	2228a	± 154	3805a	± 313	331.18b	± 30.21	3.90a	± 0.23
Nutrient		**Fe**	**St. Error**	**Mn**	**St. Error**	**Mo**	**St. Error**	**Cu**	**St. Error**	**Zn**	**St. Error**	**Se**	**St. Error**	**Co**	**St. Error**
100% Daylight	**Control**	141.29a	± 35.02	151.11a	± 41.13	2.77a	± 0.71	11.89a	± 3.57	46.55ab	± 13.91	0.035a	± 0.010	0.122a	± 0.038
(Light)	**Treated**	186.64a	± 46.9	138.87a	± 2.33	2.96a	± 0.10	11.04a	± 0.34	45.54a	± 0.97	0.038a	± 0.001	0.133a	± 0.004
50% Daylight	**Control**	237.34a	± 39.36	117.52a	± 7.43	2.73a	± 0.24	13.18a	± 0.93	68.81b	± 7.2	0.048a	± 0.007	0.223b	± 0.061
(Shade)	**Treated**	245.48a	± 4.84	132.53a	± 20.63	2.53a	± 0.09	13.57a	± 1.38	68.03b	± 5.41	0.081a	± 0.032	0.236b	± 0.070

Phosphorus (P)

P content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Phosphorus (P) mg/kg

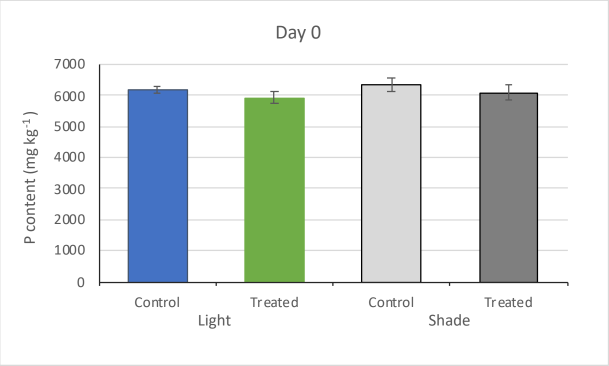
Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

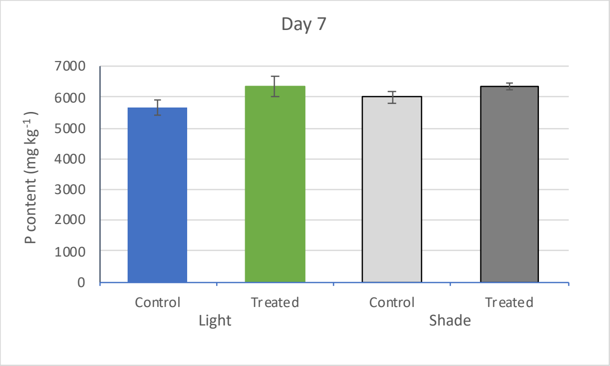
Date	Treatment	Mean		St Error
Day 0	Control Light	6188 a	±	123.70
	Treated Light	5940 a	±	170.01
	Control Shade	6346 a	±	204.38
	Treated Shade	6094 a	±	233.79
Day 7	Control Light	5665 a	±	245.81
	Treated Light	6336 a	±	332.21
	Control Shade	5993 a	±	216.37
	Treated Shade	6343 a	±	128.24
Day 14	Control Light	4893 a	±	1379.55
	Treated Light	4845 a	±	146.89
	Control Shade	5303 a	±	289.29
	Treated Shade	5725 a	±	511.26

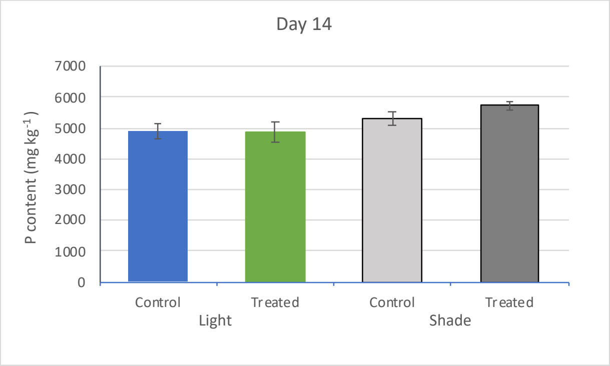
ANOVA for Phosphorus

Yellow highlight indicates significant differences at $P \leq 0.05$

Date	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day 0	ALA	1	186641.	186641.	1.77	0.220
	Light	1	73136.	73136.	0.69	0.429
	ALA.Light	1	12.	12.	0.00	0.992
	Residual	8	843782.	105473.		
	Total	11	1103571.			
Day 7	ALA	1	783360.	783360.	4.46	0.068
	Light	1	84373.	84373.	0.48	0.508
	ALA.Light	1	77231.	77231.	0.44	0.526
	Residual	8	1404265.	175533.		
	Total	11	2349229.			
Day 14	ALA	1	104874.	104874.	0.06	0.810
	Light	1	1248152.	1248152.	0.73	0.417
	ALA.Light	1	164634.	164634.	0.10	0.764
	Residual	8	13618917.	1702365.		
	Total	11	15136577.			







Potassium (K)

K content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Potassium (K) mg/kg

Means with same letters within each row or column of each data set are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	48994a	±	1545.86
	Treated Light	46790a	±	2687.13
	Control Shade	48165a	±	1567.82
	Treated Shade	47560a	±	1110.60
Day 7	Control Light	44378a	±	2230.33
	Treated Light	44071a	±	1292.11
	Control Shade	41502a	±	1461.06
	Treated Shade	43590a	±	664.66
Day 14	Control Light	36470a	±	11816.73
	Treated Light	33999a	±	210.40
	Control Shade	34854a	±	3835.86
	Treated Shade	34262a	±	4261.89

ANOVA for Potassium

Yellow highlight indicates significant differences at P≤ 0.05

Day 0

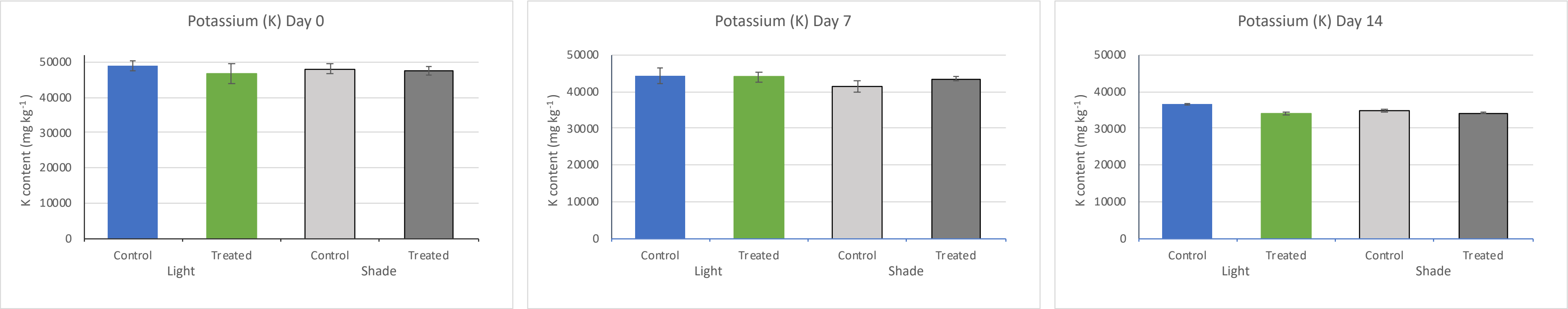
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	5914862.	5914862.	0.59	0.463
Light	1	2593.	2593.	0.00	0.988
ALA.Light	1	1918350.	1918350.	0.19	0.673
Residual	8	79810980.	9976372.		
Total	11	87646785.			

Day 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	2379153.	2379153.	0.34	0.574
Light	1	8453192.	8453192.	1.22	0.301
ALA.Light	1	4300941.	4300941.	0.62	0.453
Residual	8	55322371.	6915296.		
Total	11	70455656.			

Day 14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	7.037E+06	7.037E+06	0.05	0.821
Light	1	1.375E+06	1.375E+06	0.01	0.920
ALA.Light	1	2.647E+06	2.647E+06	0.02	0.890
Residual	8	1.035E+09	1.294E+08		
Total	11	1.046E+09			



Calcium (Ca)

Ca content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Calcium (Ca) mg/kg

Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	3979a	±	155.69
	Treated Light	4110a	±	152.01
	Control Shade	4088a	±	69.93
	Treated Shade	3946a	±	175.32
Day 7	Control Light	3635ab	±	91.01
	Treated Light	3876b	±	59.95
	Control Shade	3644ab	±	152.61
	Treated Shade	3457a	±	138.51
Day 14	Control Light	3723a	±	1211.27
	Treated Light	3172a	±	48.40
	Control Shade	3812a	±	262.65
	Treated Shade	4324a	±	712.72

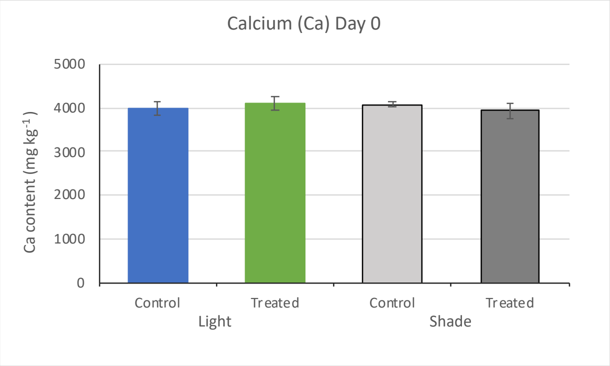
ANOVA for Calcium

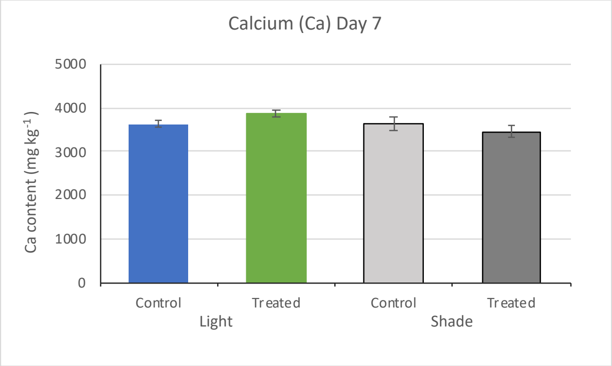
Yellow highlight indicates significant differences at $P \leq 0.05$

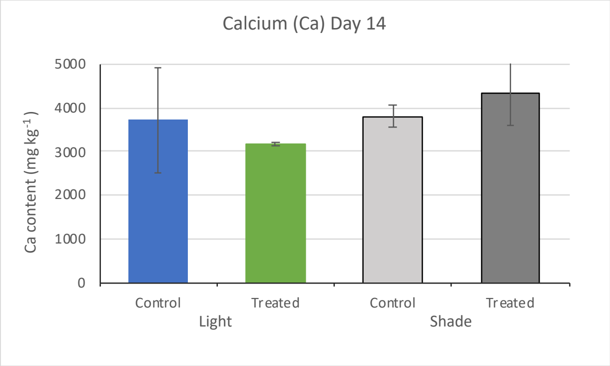
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	92.	92.	0.00	0.970
Light	1	2208.	2208.	0.04	0.855
ALA.Light	1	55933.	55933.	0.90	0.371
Residual	8	497857.	62232.		
Total	11	556090.			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	2206.	2206.	0.05	0.822
Light	1	126340.	126340.	3.10	0.116
ALA.Light	1	137158.	137158.	3.36	0.104
Residual	8	326121.	40765.		
Total	11	591825.			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	1176.	1176.	0.00	0.979
Light	1	1154889.	1154889.	0.75	0.411
ALA.Light	1	847292.	847292.	0.55	0.479
Residual	8	12278775.	1534847.		
Total	11	14282132.			







Magnesium (Mg)

Mg content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Magnesium (Mg) mg/kg

Means with same letters within each row or column of each data set are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	2679ab	±	46.04
	Treated Light	2484a	±	48.24
	Control Shade	2748b	±	9.60
	Treated Shade	2646ab	±	96.07
Day 7	Control Light	2481ab	±	28.33
	Treated Light	2538b	±	1.22
	Control Shade	2308a	±	31.09
	Treated Shade	2377ab	±	112.29
Day 14	Control Light	2371a	±	742.86
	Treated Light	2034a	±	55.65
	Control Shade	2335a	±	241.39
	Treated Shade	2228a	±	154.20

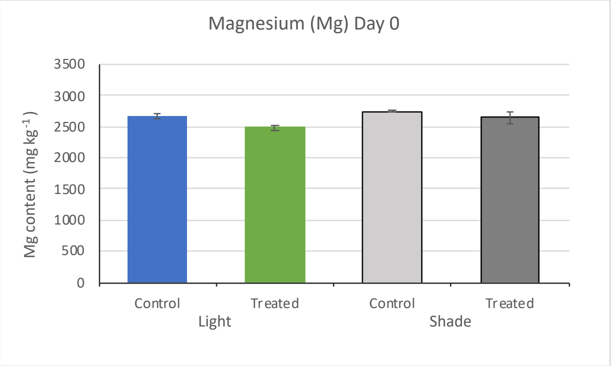
ANOVA for Calcium

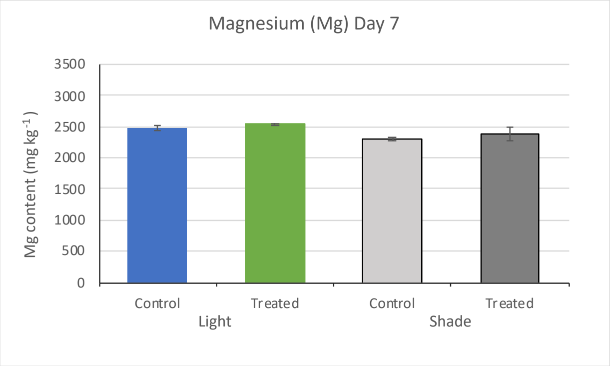
Yellow highlight indicates significant differences at P≤ 0.05

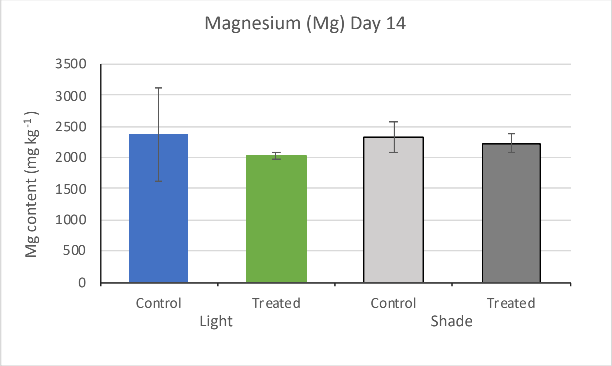
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	66434.	66434.	6.43	0.035
Light	1	39582.	39582.	3.83	0.086
ALA.Light	1	6554.	6554.	0.63	0.449
Residual	8	82618.	10327.		
Total	11	195187.			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	11828.	11828.	1.10	0.326
Light	1	83556.	83556.	7.75	0.024
ALA.Light	1	90.	90.	0.01	0.929
Residual	8	86271.	10784.		
Total	11	181745.			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	148003.	148003.	0.31	0.593
Light	1	18804.	18804.	0.04	0.848
ALA.Light	1	39439.	39439.	0.08	0.781
Residual	8	3821887.	477736.		
Total	11	4028133.			







Sulphur (S)

S content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Sulphur (S) mg/kg

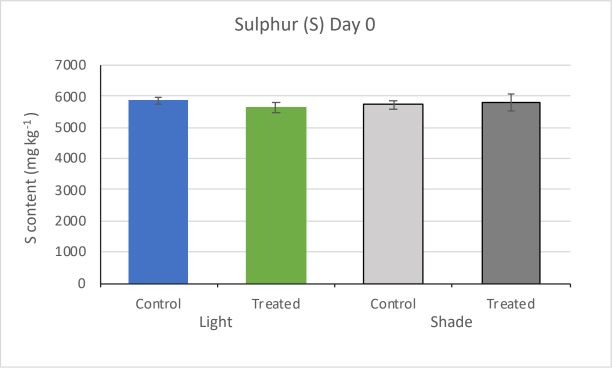
Means with same letters within each row or column of each data set are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

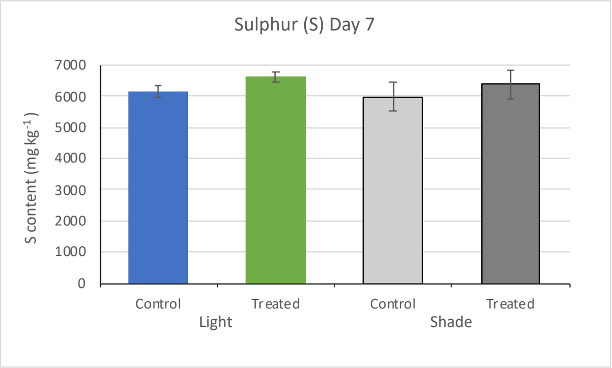
Date	Treatment	Mean		St Error
Day 0	Control Light	5872a	±	124.88
	Treated Light	5657a	±	171.85
	Control Shade	5732a	±	150.66
	Treated Shade	5810a	±	253.84
Day 7	Control Light	6162a	±	208.68
	Treated Light	6631a	±	151.30
	Control Shade	5975a	±	465.01
	Treated Shade	6385a	±	462.76
Day 14	Control Light	5445a	±	1614.78
	Treated Light	4962a	±	47.26
	Control Shade	4002a	±	244.44
	Treated Shade	3805a	±	312.78

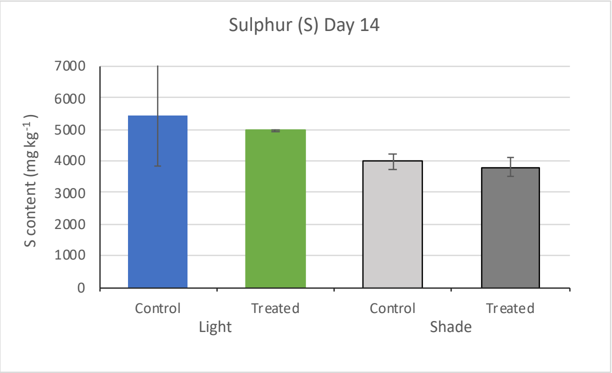
ANOVA for Sulphur

Yellow highlight indicates significant differences at P≤ 0.05

Date	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day 0	ALA	1	14154.	14154.	0.14	0.715
	Light	1	135.	135.	0.00	0.972
	ALA.Light	1	64321.	64321.	0.65	0.444
	Residual	8	793560.	99195.		
	Total	11	872169.			
Day 7	ALA	1	578502.	578502.	1.55	0.248
	Light	1	141345.	141345.	0.38	0.555
	ALA.Light	1	2665.	2665.	0.01	0.935
	Residual	8	2980936.	372617.		
	Total	11	3703448.			
Day 14	ALA	1	346697.	346697.	0.17	0.693
	Light	1	5070927.	5070927.	2.44	0.157
	ALA.Light	1	61154.	61154.	0.03	0.868
	Residual	8	16603950.	2075494.		
	Total	11	22082727.			







Sodium (Na)

S content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Sodium (Na) mg/kg

Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	162.12b	±	10.32
	Treated Light	182.50a	±	8.32
	Control Shade	182.96a	±	5.03
	Treated Shade	164.16b	±	6.08
Day 7	Control Light	212.97a	±	7.09
	Treated Light	241.86a	±	28.55
	Control Shade	234.56a	±	20.87
	Treated Shade	238.36a	±	19.48
Day 14	Control Light	190.30a	±	60.88
	Treated Light	207.76a	±	14.22
	Control Shade	330.31b	±	54.27
	Treated Shade	331.1b	±	30.21

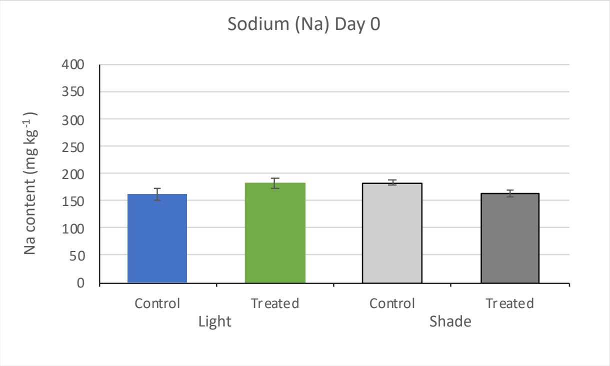
ANOVA for Sodium

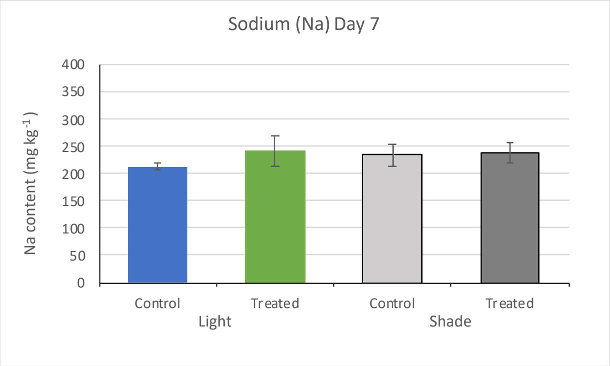
Yellow highlight indicates significant differences at $P \leq 0.05$

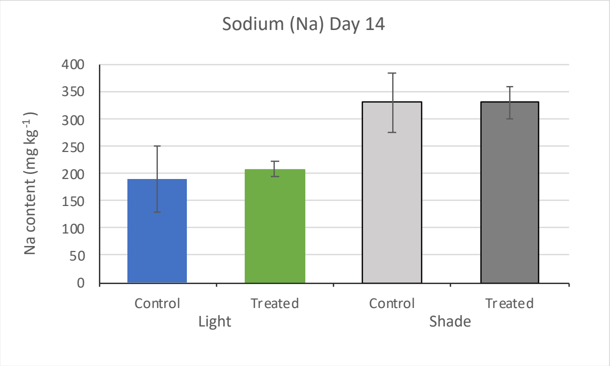
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	1.9	1.9	0.01	0.921
Light	1	4.7	4.7	0.03	0.875
ALA.Light	1	1151.1	1151.1	6.45	0.035
Residual	8	1426.8	178.4		
Total	11	2584.5			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	802.	802.	0.64	0.448
Light	1	246.	246.	0.19	0.671
ALA.Light	1	472.	472.	0.37	0.557
Residual	8	10083.	1260.		
Total	11	11603.			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	252.	252.	0.04	0.840
Light	1	52048.	52048.	8.94	0.017
ALA.Light	1	206.	206.	0.04	0.855
Residual	8	46596.	5824.		
Total	11	99103			







Boron (B)

S content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Boron (B) mg/kg

Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	6.50ab	±	0.55
	Treated Light	7.16b	±	0.48
	Control Shade	5.63ab	±	0.70
	Treated Shade	4.77a	±	0.13
Day 7	Control Light	7.46a	±	1.10
	Treated Light	10.00a	±	0.87
	Control Shade	7.21a	±	0.31
	Treated Shade	8.18a	±	0.76
Day 14	Control Light	3.69a	±	0.98
	Treated Light	3.16a	±	0.07
	Control Shade	3.79a	±	0.07
	Treated Shade	3.90a	±	0.23

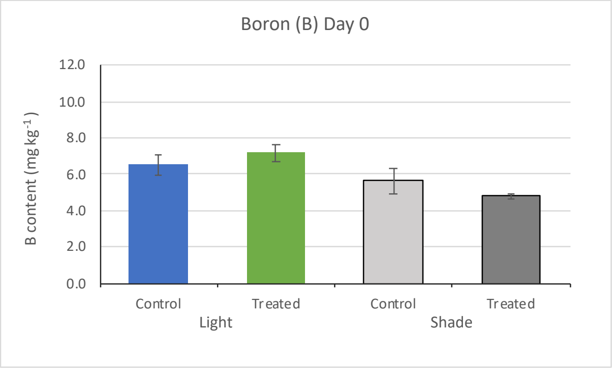
ANOVA for Boron

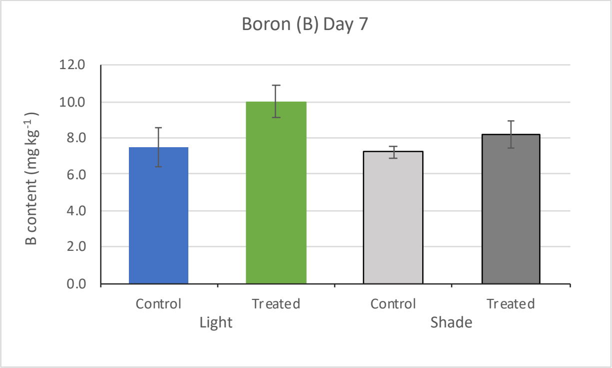
Yellow highlight indicates significant differences at $P \leq 0.05$

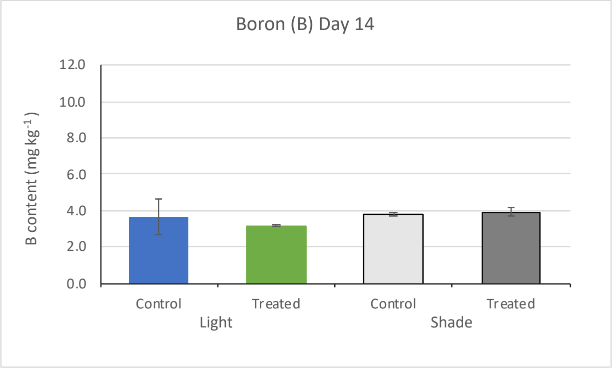
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0288	0.0288	0.04	0.852
Light	1	7.9416	7.9416	10.19	0.013
ALA.Light	1	1.7421	1.7421	2.23	0.173
Residual	8	6.2367	0.7796		
Total	11	15.9492			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	9.248	9.248	4.68	0.063
Light	1	3.208	3.208	1.62	0.239
ALA.Light	1	1.851	1.851	0.94	0.362
Residual	8	15.821	1.978		
Total	11	30.128			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.1323	0.1323	0.17	0.690
Light	1	0.5315	0.5315	0.69	0.431
ALA.Light	1	0.3020	0.3020	0.39	0.549
Residual	8	6.1845	0.7731		
Total	11	7.1504			







Iron (Fe)

Fe content (mg kg^{-1}) in turfgrass leaves (*l. perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Iron (Fe) mg/kg

Means with same letters within each row or column of each data set are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	201.24ab	±	32.08
	Treated Light	206.13b	±	24.70
	Control Shade	145.50ab	±	7.93
	Treated Shade	144.46a	±	10.72
Day 7	Control Light	190.70ab	±	24.90
	Treated Light	280.89b	±	73.82
	Control Shade	167.70ab	±	28.41
	Treated Shade	135.70a	±	6.76
Day 14	Control Light	141.29a	±	35.02
	Treated Light	186.64a	±	46.90
	Control Shade	237.34a	±	39.36
	Treated Shade	245.48a	±	4.84

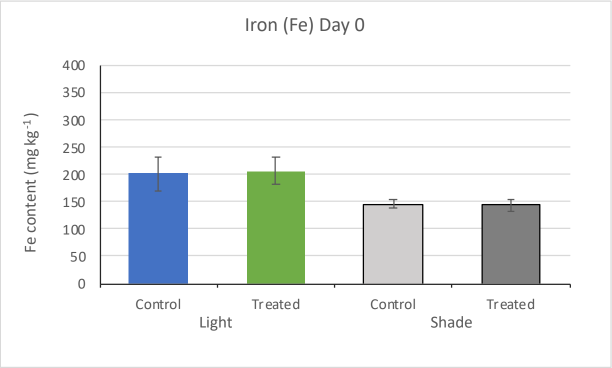
ANOVA for Iron

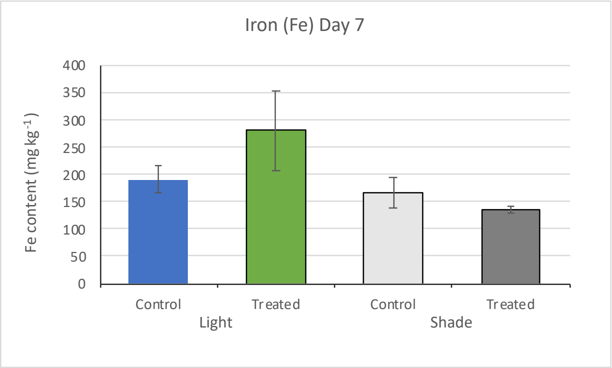
Yellow highlight indicates significant differences at P≤ 0.05

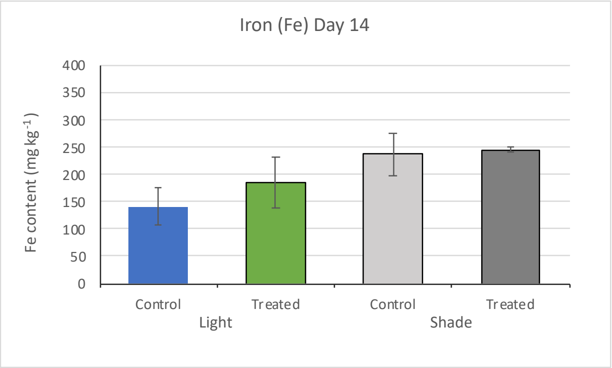
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	11.	11.	0.01	0.930
Light	1	10340.	10340.	7.59	0.025
ALA.Light	1	26.	26.	0.02	0.893
Residual	8	10903.	1363.		
Total	11	21281.			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	2540.	2540.	0.49	0.504
Light	1	21215.	21215.	4.09	0.078
ALA.Light	1	11198.	11198.	2.16	0.180
Residual	8	41532.	5191.		
Total	11	76485.			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	2146.	2146.	0.57	0.471
Light	1	17992.	17992.	4.80	0.060
ALA.Light	1	1039.	1039.	0.28	0.613
Residual	8	29991.	3749.		
Total	11	51168.			







Manganese (Mn)

Mn content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Manganese (Mn) mg/kg

Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	179.48a	±	3.01
	Treated Light	184.51a	±	7.17
	Control Shade	174.04a	±	3.22
	Treated Shade	183.58a	±	7.61
Day 7	Control Light	152.66a	±	4.44
	Treated Light	181.95b	±	2.43
	Control Shade	163.22a	±	1.88
	Treated Shade	156.76a	±	4.74
Day 14	Control Light	151.11a	±	41.13
	Treated Light	138.87a	±	2.33
	Control Shade	117.52a	±	7.43
	Treated Shade	132.53a	±	20.63

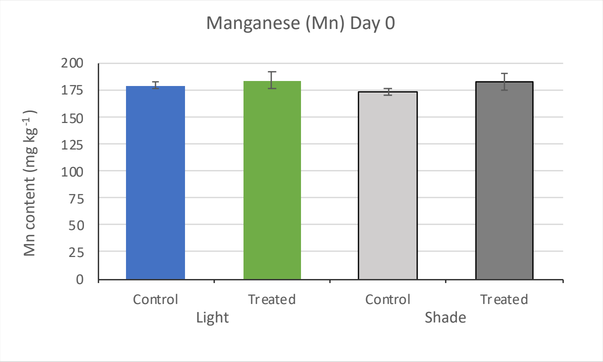
ANOVA for Manganese

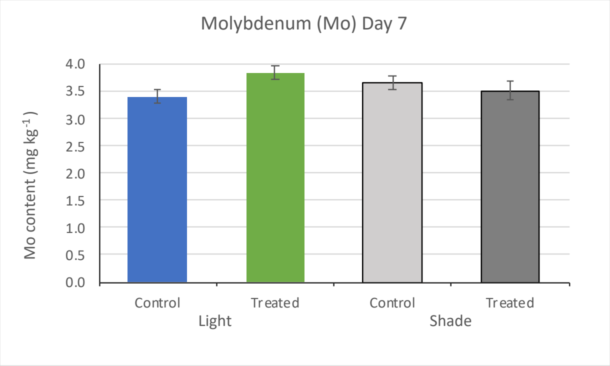
Yellow highlight indicates significant differences at $P \leq 0.05$

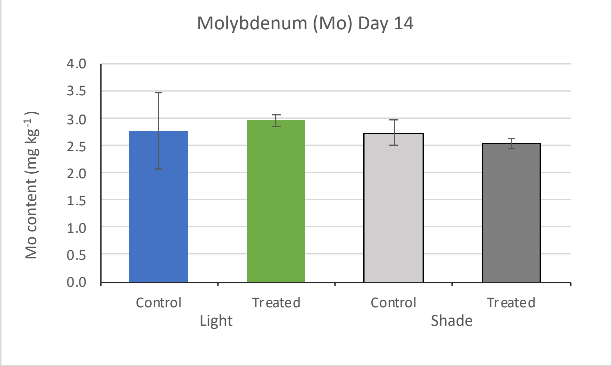
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	159.32	159.32	1.65	0.235
Light	1	30.48	30.48	0.32	0.590
ALA.Light	1	15.28	15.28	0.16	0.701
Residual	8	772.67	96.58		
Total	11	977.76			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	390.73	390.73	10.09	0.013
Light	1	160.71	160.71	4.15	0.076
ALA.Light	1	958.11	958.11	24.73	0.001
Residual	8	309.92	38.74		
Total	11	1819.46			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	6.	6.	0.00	0.954
Light	1	1195.	1195.	0.73	0.417
ALA.Light	1	557.	557.	0.34	0.575
Residual	8	13066.	1633.		
Total	11	14824.			







Molybdenum (Mo)

Mo content (mg kg^{-1}) in turfgrass leaves (*l. perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Molybdenum (Mo) mg/kg

Means with same letters within each row or column of each data set are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	3.10a	±	0.05
	Treated Light	3.09a	±	0.12
	Control Shade	3.08a	±	0.16
	Treated Shade	2.98a	±	0.25
Day 7	Control Light	3.41a	±	0.13
	Treated Light	3.84a	±	0.12
	Control Shade	3.67a	±	0.12
	Treated Shade	3.51a	±	0.18
Day 14	Control Light	2.77a	±	0.71
	Treated Light	2.96a	±	0.10
	Control Shade	2.73a	±	0.24
	Treated Shade	2.53a	±	0.09

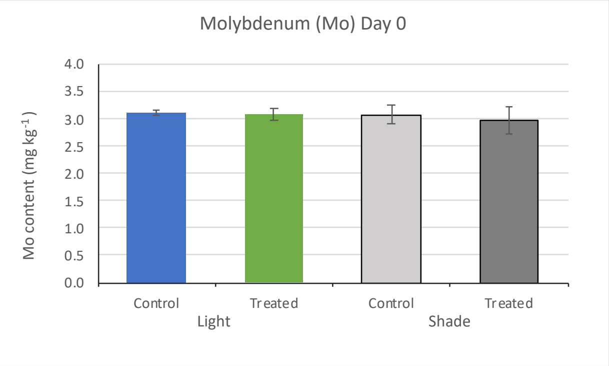
ANOVA for Molybdenum

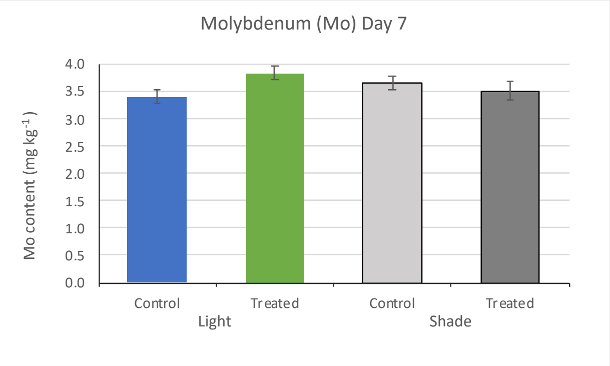
Yellow highlight indicates significant differences at P≤ 0.05

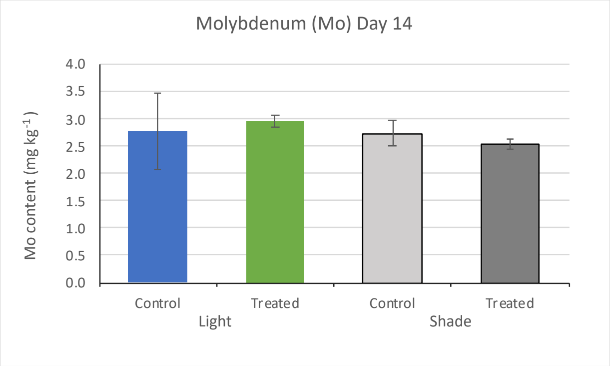
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00980	0.00980	0.12	0.736
Light	1	0.01155	0.01155	0.14	0.715
ALA.Light	1	0.00499	0.00499	0.06	0.809
Residual	8	0.64303	0.08038		
Total	11	0.66938			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.05739	0.05739	0.99	0.350
Light	1	0.00381	0.00381	0.07	0.804
ALA.Light	1	0.25592	0.25592	4.40	0.069
Residual	8	0.46536	0.05817		
Total	11	0.78249			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0001	0.0001	0.00	0.987
Light	1	0.1611	0.1611	0.37	0.559
ALA.Light	1	0.1110	0.1110	0.26	0.627
Residual	8	3.4725	0.4341		
Total	11	3.7447			







Copper (Cu)

Cu content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Copper (Cu) mg/kg

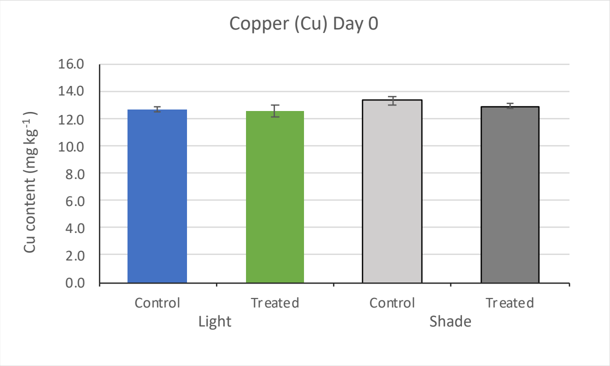
Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

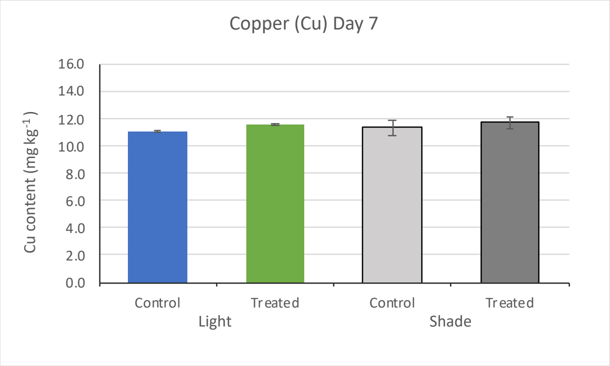
Date	Treatment	Mean		St Error
Day 0	Control Light	12.73a	±	0.16
	Treated Light	12.55a	±	0.44
	Control Shade	13.34a	±	0.29
	Treated Shade	12.92a	±	0.20
Day 7	Control Light	11.07a	±	0.10
	Treated Light	11.59a	±	0.04
	Control Shade	11.37a	±	0.57
	Treated Shade	11.73a	±	0.43
Day 14	Control Light	11.89a	±	3.57
	Treated Light	11.04a	±	0.34
	Control Shade	13.18a	±	0.93
	Treated Shade	13.57a	±	1.38

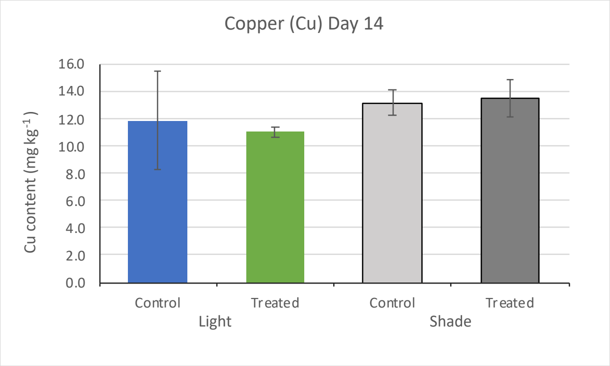
ANOVA for Copper

Yellow highlight indicates significant differences at $P \leq 0.05$

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.2674	0.2674	1.05	0.336
Light	1	0.7174	0.7174	2.81	0.132
ALA.Light	1	0.0432	0.0432	0.17	0.692
Residual	8	2.0423	0.2553		
Total	11	3.0703			
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.5673	0.5673	1.44	0.264
Light	1	0.1413	0.1413	0.36	0.566
ALA.Light	1	0.0195	0.0195	0.05	0.829
Residual	8	3.1497	0.3937		
Total	11	3.8778			
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.16	0.16	0.01	0.909
Light	1	10.89	10.89	0.93	0.363
ALA.Light	1	1.16	1.16	0.10	0.761
Residual	8	93.66	11.71		
Total	11	105.88			







Zinc (Zn)

Zn content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.) grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Zinc (Zn) mg/kg

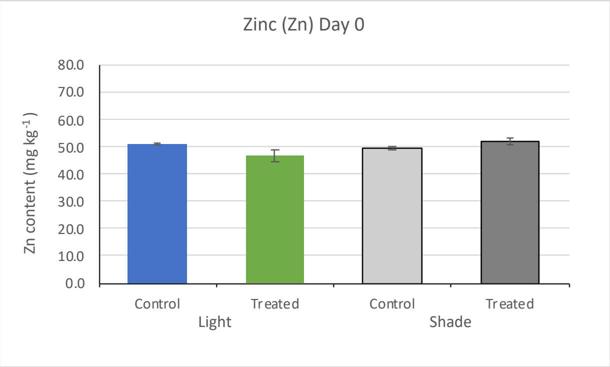
Means with same letters within each row or column of each data set are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

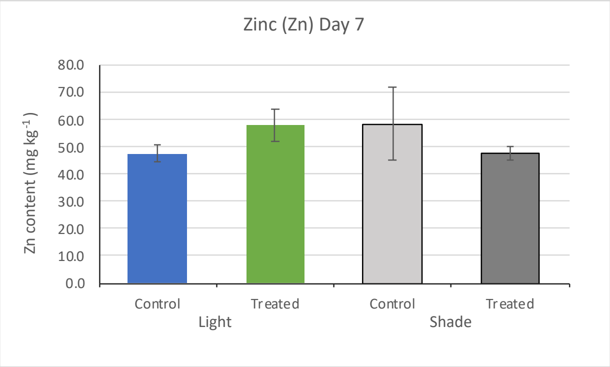
Date	Treatment	Mean		St Error
Day 0	Control Light	51.13b	±	0.39
	Treated Light	46.81a	±	2.06
	Control Shade	49.66ab	±	0.68
	Treated Shade	52.08b	±	1.19
Day 7	Control Light	47.49a	±	3.23
	Treated Light	57.86a	±	5.91
	Control Shade	58.54a	±	13.41
	Treated Shade	47.87a	±	2.39
Day 14	Control Light	46.55ab	±	13.91
	Treated Light	45.54a	±	0.97
	Control Shade	68.81b	±	7.20
	Treated Shade	68.03b	±	5.41

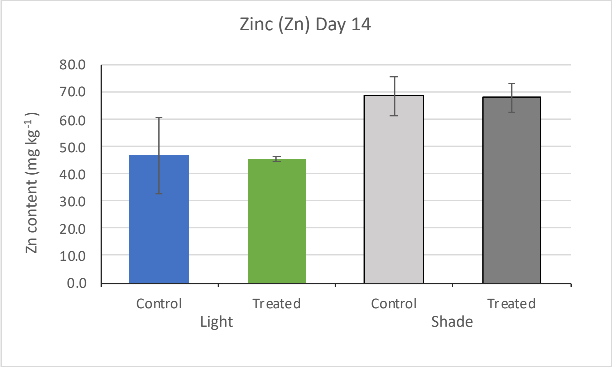
ANOVA for Zinc

Yellow highlight indicates significant differences at P≤ 0.05

Date	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day 0	ALA	1	2.705	2.705	0.58	0.470
	Light	1	10.834	10.834	2.31	0.167
	ALA.Light	1	34.075	34.075	7.26	0.027
	Residual	8	37.558	4.695		
	Total	11	85.173			
Day 7	ALA	1	0.1	0.1	0.00	0.985
	Light	1	0.9	0.9	0.00	0.946
	ALA.Light	1	332.0	332.0	1.92	0.203
	Residual	8	1384.9	173.1		
	Total	11	1717.9			
Day 14	ALA	1	2.4	2.4	0.01	0.917
	Light	1	1502.2	1502.2	7.27	0.027
	ALA.Light	1	0.0	0.0	0.00	0.989
	Residual	8	1653.7	206.7		
	Total	11	3158.3			







Selenium (Se)

Se content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Selenium (Se) mg/kg

Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	0.047a	±	0.0019
	Treated Light	0.046a	±	0.0016
	Control Shade	0.048a	±	0.0022
	Treated Shade	0.043a	±	0.0019
Day 7	Control Light	0.057a	±	0.0049
	Treated Light	0.066a	±	0.0075
	Control Shade	0.062a	±	0.0082
	Treated Shade	0.057a	±	0.0007
Day 14	Control Light	0.035a	±	0.0102
	Treated Light	0.038a	±	0.0007
	Control Shade	0.048a	±	0.0073
	Treated Shade	0.081a	±	0.0323

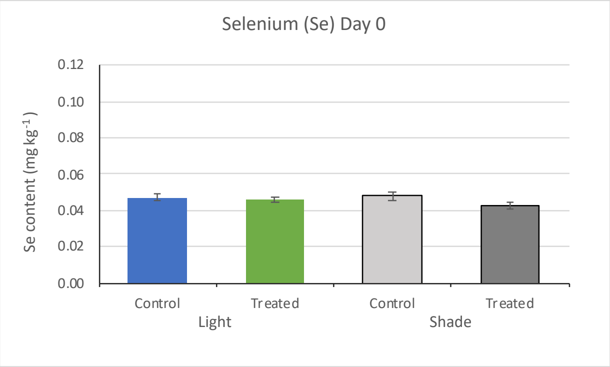
ANOVA for Selenium

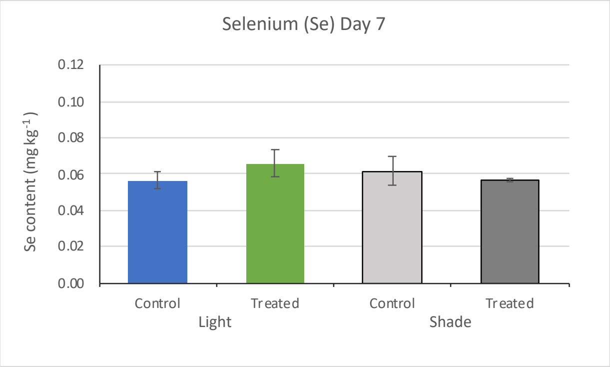
Yellow highlight indicates significant differences at $P \leq 0.05$

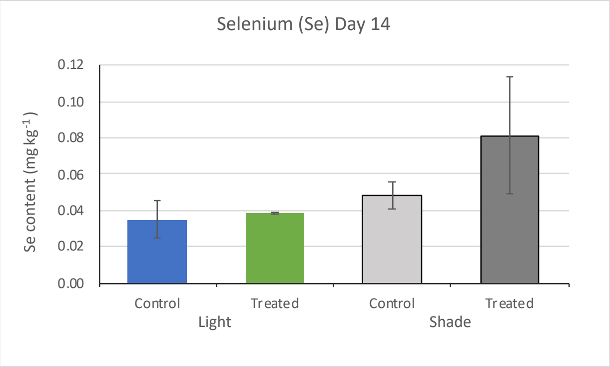
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00002465	0.00002465	2.25	0.172
Light	1	0.00000488	0.00000488	0.44	0.524
ALA.Light	1	0.00001096	0.00001096	1.00	0.347
Residual	8	0.00008775	0.00001097		
Total	11	0.00012823			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000118	0.0000118	0.11	0.753
Light	1	0.0000105	0.0000105	0.09	0.766
ALA.Light	1	0.0001460	0.0001460	1.31	0.285
Residual	8	0.0008892	0.0001111		
Total	11	0.0010575			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0009941	0.0009941	1.11	0.324
Light	1	0.0023664	0.0023664	2.63	0.143
ALA.Light	1	0.0006475	0.0006475	0.72	0.421
Residual	8	0.0071951	0.0008994		
Total	11	0.0112031			







Cobalt (Co)

Se content (mg kg^{-1}) in turfgrass leaves (*L. perenne* L.*)* grown in 100% Daylight (Light) and 50% Daylight (Shade), Days 0, 7 & 14 after treatment with 100mg L^{-1} 5-aminolevulinic acid. Bars show Standard Error of the Mean.

Cobalt (Co) mg/kg

Means with same letters within each row or column of each data set are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

Date	Treatment	Mean		St Error
Day 0	Control Light	0.112a	±	0.0010
	Treated Light	0.122a	±	0.0058
	Control Shade	0.112a	±	0.0024
	Treated Shade	0.117a	±	0.0037
Day 7	Control Light	0.094a	±	0.0027
	Treated Light	0.125b	±	0.0102
	Control Shade	0.105ab	±	0.0068
	Treated Shade	0.091a	±	0.0066
Day 14	Control Light	0.122a	±	0.0379
	Treated Light	0.133a	±	0.0044
	Control Shade	0.223a	±	0.0610
	Treated Shade	0.236a	±	0.0704

ANOVA for Cobalt

Yellow highlight indicates significant differences at $P \leq 0.05$

Day 0:

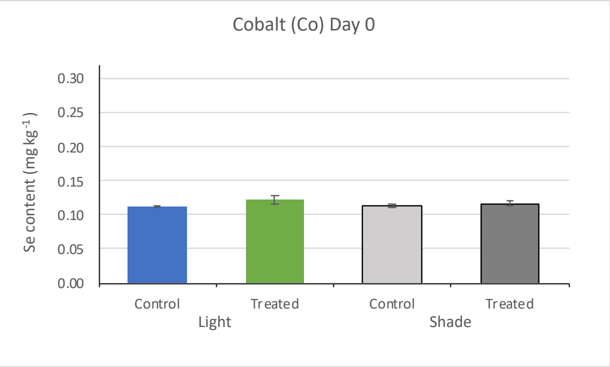
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00015795	0.00015795	3.95	0.082
Light	1	0.00002127	0.00002127	0.53	0.486
ALA.Light	1	0.00001912	0.00001912	0.48	0.509
Residual	8	0.00031953	0.00003994		
Total	11	0.00051786			

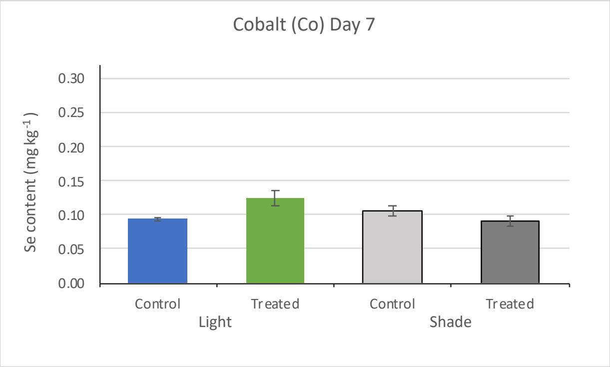
Day 7:

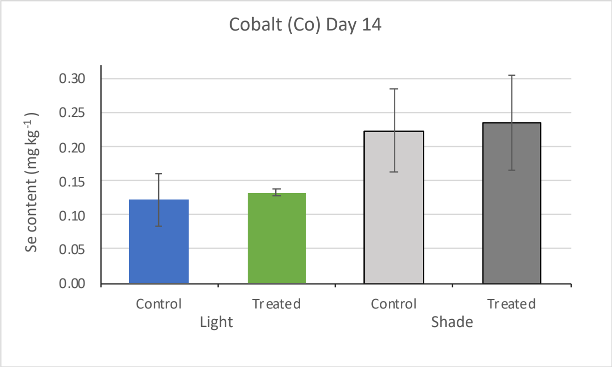
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0002113	0.0002113	1.40	0.271
Light	1	0.0003555	0.0003555	2.35	0.164
ALA.Light	1	0.0015472	0.0015472	10.23	0.013
Residual	8	0.0012105	0.0001513		
Total	11	0.0033246			

Day 14:

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000404	0.000404	0.05	0.823
Light	1	0.031261	0.031261	4.12	0.077
ALA.Light	1	0.000006	0.000006	0.00	0.979
Residual	8	0.060763	0.007595		
Total	11	0.092434			







APPENDIX V
Results of Chlorophyll *a* Fluorescence

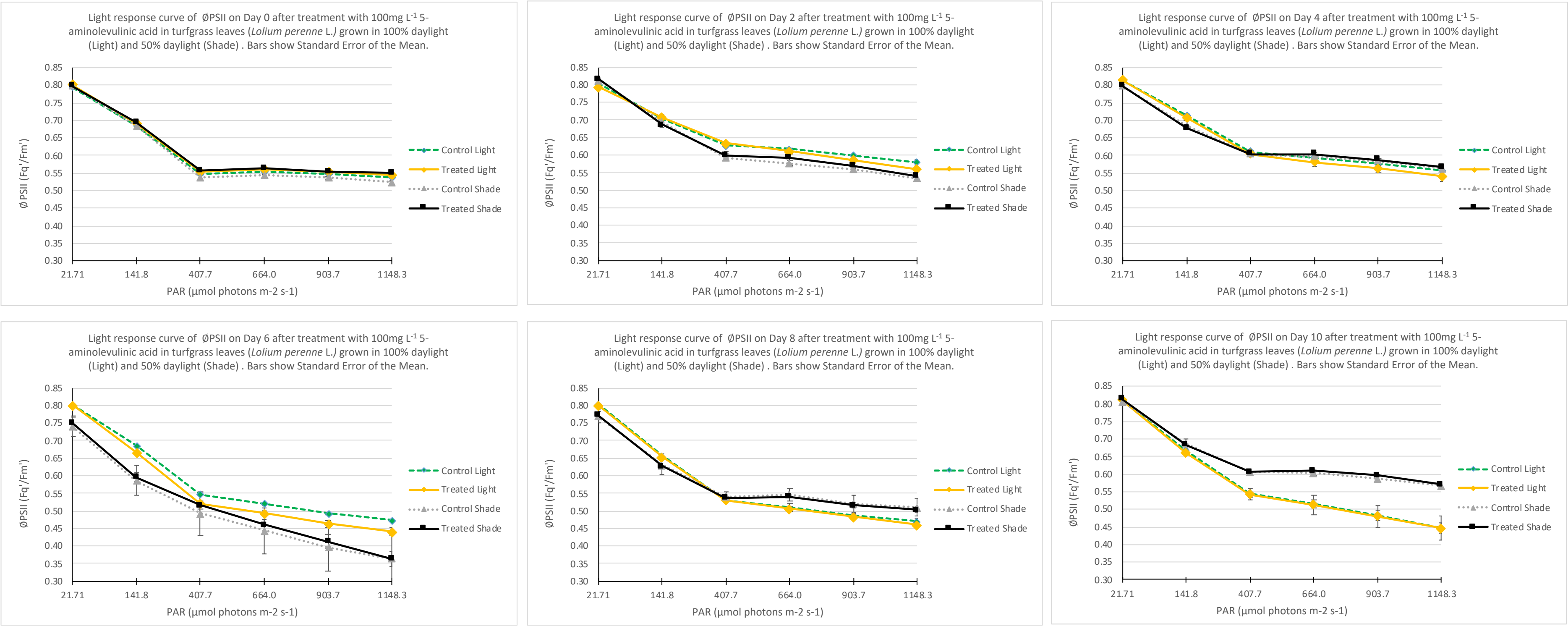
PSII Operating Efficiency (ØPSII Fq'/Fm')

Means with same letters within datasets Days After Treatment and PPFD level are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

	% of Full Daylight	**10.00%**		**20.00%**		**40.00%**		**60.00%**		**80.00%**		**100.00%**	
	PPFD (µmol photons m-2s-1)	**21.71**		**141.80**		**407.70**		**664.00**		**903.70**		**1148.30**	
Days after treatment	**Treated/light**	**Mean ØPSII**	**Standard Error**	**Mean ØPSII**	**Standard Error**	**Mean ØPSII**	**Standard Error**	**Mean ØPSII**	**Standard Error**	**Mean ØPSII**	**Standard Error**	**Mean ØPSII**	**Standard Error**
Day 0	Control Light	0.79a	± 0.0033	0.68a	± 0.0067	0.55a	± 0.0033	0.55a	± 0.0088	0.55a	± 0.0067	0.54ab	± 0.0067
	Treated Light	0.80a	± 0.0058	0.69a	± 0.0000	0.55a	± 0.0067	0.56a	± 0.0058	0.55a	± 0.0067	0.54b	± 0.0033
	Control Shade	0.80a	± 0.0033	0.68a	± 0.0088	0.54a	± 0.0088	0.54a	± 0.0067	0.54a	± 0.0033	0.52a	± 0.0033
	Treated Shade	0.80a	± 0.0033	0.69a	± 0.0033	0.56a	± 0.0033	0.56a	± 0.0033	0.55a	± 0.0033	0.55b	± 0.0058
Day 2	Control Light	0.80a	± 0.0033	0.70a	± 0.0033	0.63b	± 0.0033	0.62c	± 0.0033	0.60c	± 0.0000	0.58c	± 0.0058
	Treated Light	0.79ab	± 0.0033	0.71a	± 0.0088	0.63b	± 0.0033	0.61bc	± 0.0058	0.59bc	± 0.0088	0.56bc	± 0.0100
	Control Shade	0.81bc	± 0.0033	0.69a	± 0.0067	0.59a	± 0.0088	0.58a	± 0.0067	0.56a	± 0.0058	0.53a	± 0.0033
	Treated Shade	0.82c	± 0.0033	0.69a	± 0.0088	0.60a	± 0.0100	0.59ab	± 0.0088	0.57ab	± 0.0100	0.54ab	± 0.0100
Day 4	Control Light	0.81b	± 0.0033	0.71b	± 0.0067	0.61a	± 0.0058	0.59ab	± 0.0033	0.58a	± 0.0033	0.56a	± 0.0033
	Treated Light	0.81b	± 0.0033	0.71b	± 0.0088	0.60a	± 0.0067	0.58a	± 0.0100	0.56a	± 0.0120	0.54a	± 0.0153
	Control Shade	0.80a	± 0.0033	0.68a	± 0.0033	0.60a	± 0.0033	0.60ab	± 0.0033	0.58a	± 0.0067	0.56a	± 0.0067
	Treated Shade	0.80a	± 0.0033	0.68a	± 0.0033	0.60a	± 0.0033	0.60b	± 0.0033	0.59a	± 0.0033	0.57a	± 0.0033
Day 6	Control Light	0.80b	± 0.0000	0.69c	± 0.0033	0.55a	± 0.0033	0.52a	± 0.0058	0.49a	± 0.0033	0.47a	± 0.0033
	Treated Light	0.80ab	± 0.0000	0.67bc	± 0.0033	0.52a	± 0.0058	0.49a	± 0.0088	0.46a	± 0.0088	0.44a	± 0.0115
	Control Shade	0.74a	± 0.0300	0.59a	± 0.0433	0.49a	± 0.0617	0.44a	± 0.0667	0.40a	± 0.0684	0.36a	± 0.0669
	Treated Shade	0.75ab	± 0.0173	0.60ab	± 0.0120	0.52a	± 0.0120	0.46a	± 0.0153	0.41a	± 0.0203	0.36a	± 0.0203
Day 8	Control Light	0.80a	± 0.0033	0.66a	± 0.0067	0.53a	± 0.0058	0.51ab	± 0.0100	0.49a	± 0.0067	0.47a	± 0.0100
	Treated Light	0.80a	± 0.0000	0.65a	± 0.0033	0.53a	± 0.0058	0.51a	± 0.0067	0.48a	± 0.0088	0.46a	± 0.0115
	Control Shade	0.77a	± 0.0200	0.63a	± 0.0252	0.54a	± 0.0153	0.55a	± 0.0186	0.52a	± 0.0252	0.51a	± 0.0252
	Treated Shade	0.77a	± 0.0088	0.63a	± 0.0033	0.54a	± 0.0033	0.54ab	± 0.0000	0.52a	± 0.0067	0.50	± 0.0088
Day 10	Control Light	0.81a	± 0.0000	0.67a	± 0.0067	0.55a	± 0.0120	0.52a	± 0.0120	0.48a	± 0.0145	0.45a	± 0.0145
	Treated Light	0.81a	± 0.0000	0.66a	± 0.0058	0.54a	± 0.0167	0.51a	± 0.0273	0.48a	± 0.0306	0.45a	± 0.0338
	Control Shade	0.81a	± 0.0088	0.69a	± 0.0133	0.61b	± 0.0033	0.60b	± 0.0067	0.59b	± 0.0088	0.57b	± 0.0088
	Treated Shade	0.81a	± 0.0033	0.68a	± 0.0033	0.61b	± 0.0033	0.61b	± 0.0000	0.60b	± 0.0033	0.57b	± 0.0000

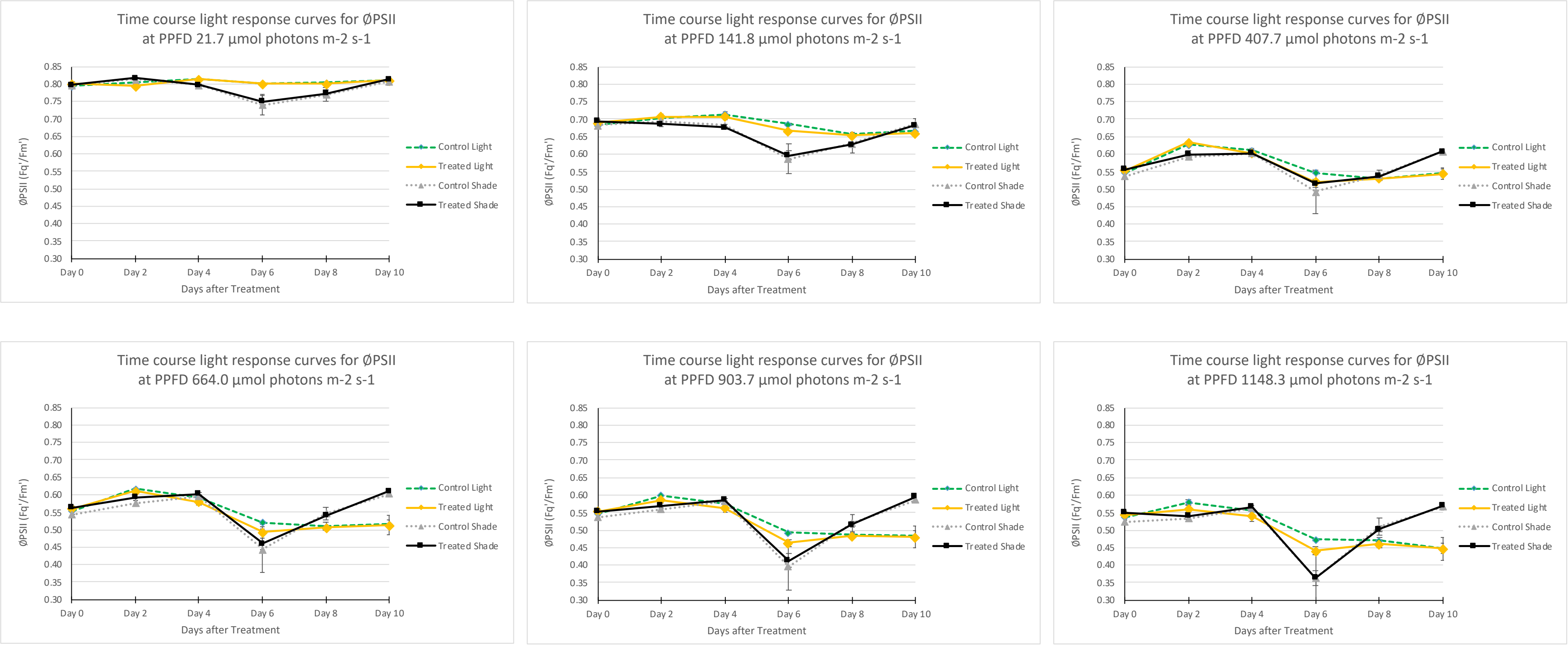
Light response curves for PSII Operating Efficiency (ØPSII Fq'/Fm')

Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)



Time course response curves for PSII Operating Efficiency (ØPSII Fq'/Fm')

Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)



Time course light response curves for ØPSII at PPFD 21.7 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 141.8 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 407.7 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 664.0 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 903.7 µmol photons m-2 s-1

Time course light response curves for ØPSII at PPFD 1148.3 µmol photons m-2 s-1

ANOVA for PSII Operating Efficiency (ØPSII Fq'/Fm')

DAY 0

Variate: QY_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	0.67	0.438
Light	1	0.00000000	0.00000000	0.00	1.000
ALA.Light	1	0.00003333	0.00003333	0.67	0.438
Residual	8	0.00040000	0.00005000		
Total	11	0.00046667			

Variate: QY_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0002083	0.0002083	2.08	0.187
Light	1	0.0000083	0.0000083	0.08	0.780
ALA.Light	1	0.0000083	0.0000083	0.08	0.780
Residual	8	0.0008000	0.0001000		
Total	11	0.0010250			

Variate: QY_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

ALA	1	0.0005333	0.0005333	4.92	0.057
Light	1	0.0000333	0.0000333	0.31	0.594
ALA.Light	1	0.0001333	0.0001333	1.23	0.299
Residual	8	0.0008667	0.0001083		
Total	11	0.0015667			

Variate: QY_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0005333	0.0005333	4.27	0.073
Light	1	0.0000333	0.0000333	0.27	0.620
ALA.Light	1	0.0001333	0.0001333	1.07	0.332
Residual	8	0.0010000	0.0001250		
Total	11	0.0017000			

Variate: QY_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00040833	0.00040833	4.90	0.058
Light	1	0.00007500	0.00007500	0.90	0.371
ALA.Light	1	0.00007500	0.00007500	0.90	0.371
Residual	8	0.00066667	0.00008333		
Total	11	0.00122500			

Variate: QY_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00083333	0.00083333	11.11	0.010
Light	1	0.00003333	0.00003333	0.44	0.524
ALA.Light	1	0.00030000	0.00030000	4.00	0.081
Residual	8	0.00060000	0.00007500		
Total	11	0.00176667			

DAY 2

Variate: QY_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	1.00	0.347
Light	1	0.00083333	0.00083333	25.00	0.001
ALA.Light	1	0.00013333	0.00013333	4.00	0.081
Residual	8	0.00026667	0.00003333		
Total	11	0.00126667			

Variate: QY_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.05	0.824
Light	1	0.0006750	0.0006750	4.26	0.073
ALA.Light	1	0.0000750	0.0000750	0.47	0.511
Residual	8	0.0012667	0.0001583		
Total	11	0.0020250			

Variate: QY_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

ALA	1	0.0001333	0.0001333	0.89	0.373
Light	1	0.0033333	0.0033333	22.22	0.002
ALA.Light	1	0.0000000	0.0000000	0.00	1.000
Residual	8	0.0012000	0.0001500		
Total	11	0.0046667			

Variate: QY_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.60	0.461
Light	1	0.0024083	0.0024083	19.27	0.002
ALA.Light	1	0.0004083	0.0004083	3.27	0.108
Residual	8	0.0010000	0.0001250		
Total	11	0.0038917			

Variate: QY_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.05	0.824
Light	1	0.0024083	0.0024083	15.21	0.005
ALA.Light	1	0.0004083	0.0004083	2.58	0.147
Residual	8	0.0012667	0.0001583		
Total	11	0.0040917			

Variate: QY_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0001333	0.0001333	0.73	0.419
Light	1	0.0033333	0.0033333	18.18	0.003
ALA.Light	1	0.0005333	0.0005333	2.91	0.126
Residual	8	0.0014667	0.0001833		
Total	11	0.0054667			

DAY 4

Variate: QY_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000000	0.00000000	0.00	1.000
Light	1	0.00083333	0.00083333	25.00	0.001
ALA.Light	1	0.00000000	0.00000000	0.00	1.000
Residual	8	0.00026667	0.00003333		
Total	11	0.00110000			

Variate: QY_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0001333	0.0001333	1.23	0.299
Light	1	0.0027000	0.0027000	24.92	0.001
ALA.Light	1	0.0000000	0.0000000	0.00	1.000
Residual	8	0.0008667	0.0001083		
Total	11	0.0037000			

Variate: QY_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	0.44	0.524
Light	1	0.00003333	0.00003333	0.44	0.524
ALA.Light	1	0.00003333	0.00003333	0.44	0.524
Residual	8	0.00060000	0.00007500		
Total	11	0.00070000			

Variate: QY_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.33	0.580
Light	1	0.0005333	0.0005333	5.33	0.050
ALA.Light	1	0.0003000	0.0003000	3.00	0.122
Residual	8	0.0008000	0.0001000		
Total	11	0.0016667			

Variate: QY_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.47	0.511
Light	1	0.0006750	0.0006750	4.26	0.073
ALA.Light	1	0.0002083	0.0002083	1.32	0.284
Residual	8	0.0012667	0.0001583		
Total	11	0.0022250			

Variate: QY_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0001333	0.0001333	0.59	0.464
Light	1	0.0008333	0.0008333	3.70	0.090
ALA.Light	1	0.0003000	0.0003000	1.33	0.282
Residual	8	0.0018000	0.0002250		
Total	11	0.0030667			

DAY 6

Variate: QY_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.08	0.780
Light	1	0.0090750	0.0090750	10.08	0.013
ALA.Light	1	0.0000750	0.0000750	0.08	0.780
Residual	8	0.0072000	0.0009000		
Total	11	0.0164250			

Variate: QY_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000075	0.000075	0.05	0.831
Light	1	0.021675	0.021675	14.14	0.006
ALA.Light	1	0.000675	0.000675	0.44	0.526
Residual	8	0.012267	0.001533		
Total	11	0.034692			

Variate: QY_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000008	0.000008	0.00	0.959
Light	1	0.002408	0.002408	0.80	0.396
ALA.Light	1	0.001875	0.001875	0.62	0.452
Residual	8	0.024000	0.003000		
Total	11	0.028292			

Variate: QY_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000075	0.000075	0.02	0.889
Light	1	0.009075	0.009075	2.53	0.151
ALA.Light	1	0.001408	0.001408	0.39	0.549
Residual	8	0.028733	0.003592		
Total	11	0.039292			

Variate: QY_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000133	0.000133	0.03	0.858
Light	1	0.016133	0.016133	4.15	0.076
ALA.Light	1	0.001633	0.001633	0.42	0.535
Residual	8	0.031067	0.003883		
Total	11	0.048967			

Variate: QY_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000833	0.000833	0.22	0.651
Light	1	0.026133	0.026133	6.92	0.030
ALA.Light	1	0.000833	0.000833	0.22	0.651
Residual	8	0.030200	0.003775		
Total	11	0.058000			

DAY 8

Variate: QY_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000000	0.0000000	0.00	1.000
Light	1	0.0027000	0.0027000	7.36	0.027
ALA.Light	1	0.0000333	0.0000333	0.09	0.771
Residual	8	0.0029333	0.0003667		
Total	11	0.0056667			

Variate: QY_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.06	0.807
Light	1	0.0021333	0.0021333	4.06	0.079
ALA.Light	1	0.0000000	0.0000000	0.00	1.000
Residual	8	0.0042000	0.0005250		
Total	11	0.0063667			

Variate: QY_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.04	0.855
Light	1	0.0002083	0.0002083	0.89	0.372
ALA.Light	1	0.0000083	0.0000083	0.04	0.855
Residual	8	0.0018667	0.0002333		
Total	11	0.0020917			

Variate: QY_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.20	0.663
Light	1	0.0036750	0.0036750	10.02	0.013
ALA.Light	1	0.0000083	0.0000083	0.02	0.884
Residual	8	0.0029333	0.0003667		
Total	11	0.0066917			

Variate: QY_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.06	0.820
Light	1	0.0033333	0.0033333	5.56	0.046
ALA.Light	1	0.0000000	0.0000000	0.00	1.000
Residual	8	0.0048000	0.0006000		
Total	11	0.0081667			

Variate: QY_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0002083	0.0002083	0.29	0.602
Light	1	0.0052083	0.0052083	7.35	0.027
ALA.Light	1	0.0000083	0.0000083	0.01	0.916
Residual	8	0.0056667	0.0007083		
Total	11	0.0110917			

DAY 10

Variate: QY_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	0.50	0.500
Light	1	0.00000000	0.00000000	0.00	1.000
ALA.Light	1	0.00003333	0.00003333	0.50	0.500
Residual	8	0.00053333	0.00006667		
Total	11	0.00060000			

Variate: QY_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.37	0.557
Light	1	0.0014083	0.0014083	7.04	0.029
ALA.Light	1	0.0000083	0.0000083	0.04	0.843
Residual	8	0.0016000	0.0002000		
Total	11	0.0030917			

Variate: QY_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.02	0.878
Light	1	0.0114083	0.0114083	34.22	<.001
ALA.Light	1	0.0000083	0.0000083	0.02	0.878
Residual	8	0.0026667	0.0003333		
Total	11	0.0140917			

Variate: QY_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.01	0.916
Light	1	0.0252083	0.0252083	36.01	<.001
ALA.Light	1	0.0000750	0.0000750	0.11	0.752
Residual	8	0.0056000	0.0007000		
Total	11	0.0308917			

Variate: QY_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.04	0.854
Light	1	0.0363000	0.0363000	39.24	<.001
ALA.Light	1	0.0001333	0.0001333	0.14	0.714
Residual	8	0.0074000	0.0009250		
Total	11	0.0438667			

Variate: QY_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000008	0.000008	0.01	0.932
Light	1	0.044408	0.044408	41.31	<.001
ALA.Light	1	0.000008	0.000008	0.01	0.932
Residual	8	0.008600	0.001075		
Total	11	0.053025			

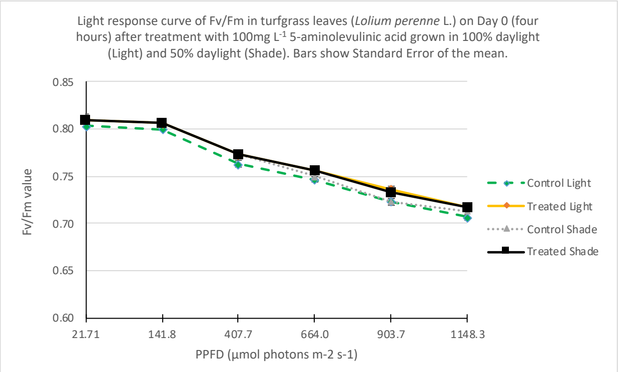
Maximum Quantum Efficiency of PSII Photochemistry (F_v/F_m)

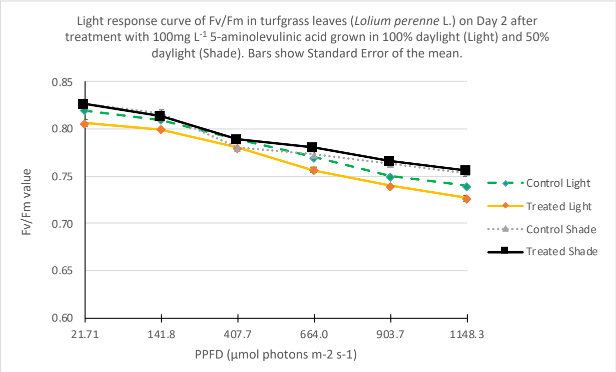
Means with same letters within data sets Days After Treatment are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

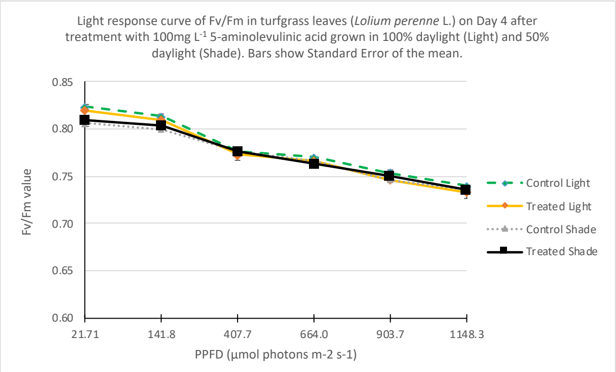
Days after treament	% of Full Daylight	10.00%		20.00%		40.00%		60.00%		80.00%		100.00%	
	PPFD (µmol photons m-2s-1)	21.71		141.80		407.70		664.00		903.70		1148.30	
	Treated/light	Mean Fv/Fm	St. Error	Mean Fv/Fm	St. Error	Mean Fv/Fm	St. Error	Mean Fv/Fm	St. Error	Mean Fv/Fm	St. Error	Mean Fv/Fm	St. Error
Day 0	Control Light	0.80a	0.0033	0.80a	0.0000	0.76a	0.0033	0.75a	0.0033	0.72a	0.0033	0.71a	0.0033
	Treated Light	0.81a	0.0058	0.81a	0.0033	0.77a	0.0033	0.76b	0.0033	0.74b	0.0033	0.72a	0.0033
	Control Shade	0.81a	0.0000	0.81a	0.0033	0.77a	0.0033	0.75ab	0.0000	0.72a	0.0033	0.71a	0.0033
	Treated Shade	0.81a	0.0000	0.81a	0.0033	0.77a	0.0033	0.76ab	0.0033	0.73ab	0.0033	0.72a	0.0033
Day 2	Control Light	0.82b	0.0000	0.81b	0.0000	0.79a	0.0000	0.77a	0.0000	0.75b	0.0000	0.74b	0.0000
	Treated Light	0.81ba	0.0033	0.80a	0.0000	0.78a	0.0000	0.76a	0.0033	0.74a	0.0000	0.73a	0.0033
	Control Shade	0.83b	0.0033	0.82b	0.0033	0.78a	0.0000	0.77bc	0.0033	0.76c	0.0033	0.75c	0.0033
	Treated Shade	0.83b	0.0033	0.81b	0.0033	0.79a	0.0000	0.78c	0.0000	0.77c	0.0033	0.76c	0.0033
Day 4	Control Light	0.82b	0.0033	0.81c	0.0033	0.78a	0.0033	0.77a	0.0000	0.75a	0.0033	0.74a	0.0000
	Treated Light	0.82b	0.0000	0.81bc	0.0000	0.77a	0.0067	0.77a	0.0033	0.75a	0.0033	0.73a	0.0067
	Control Shade	0.81a	0.0033	0.80a	0.0000	0.78a	0.0033	0.77a	0.0033	0.75a	0.0033	0.74a	0.0033
	Treated Shade	0.81a	0.0000	0.80ab	0.0033	0.78a	0.0033	0.76a	0.0033	0.75a	0.0058	0.74a	0.0033
Day 6	Control Light	0.81b	0.0033	0.80b	0.0000	0.74a	0.0033	0.74b	0.0033	0.73b	0.0033	0.72b	0.0000
	Treated Light	0.81b	0.0033	0.80b	0.0033	0.74a	0.0033	0.73b	0.0033	0.72b	0.0033	0.71b	0.0058
	Control Shade	0.75a	0.0267	0.74a	0.0285	0.70a	0.0318	0.68a	0.0318	0.66a	0.0285	0.64a	0.0273
	Treated Shade	0.77ab	0.0145	0.75ab	0.0176	0.72a	0.0176	0.69a	0.0153	0.67a	0.0167	0.64a	0.0145
Day 8	Control Light	0.82b	0.0033	0.80a	0.0033	0.73a	0.0033	0.73a	0.0058	0.72a	0.0033	0.71a	0.0058
	Treated Light	0.81b	0.0033	0.79a	0.0033	0.73a	0.0033	0.72a	0.0033	0.71a	0.0033	0.70a	0.0033
	Control Shade	0.78a	0.0186	0.76a	0.0252	0.71a	0.0267	0.71a	0.0252	0.69a	0.0252	0.68a	0.0252
	Treated Shade	0.78ab	0.0088	0.77a	0.0120	0.72a	0.0120	0.71a	0.0120	0.69a	0.0133	0.68a	0.0100
Day 10	Control Light	0.82a	0.0000	0.80a	0.0033	0.74a	0.0033	0.74b	0.0033	0.73ab	0.0033	0.72ab	0.0033
	Treated Light	0.82a	0.0000	0.80ab	0.0033	0.74a	0.0058	0.74b	0.0100	0.73a	0.0100	0.72a	0.0088
	Control Shade	0.82a	0.0058	0.81c	0.0067	0.79b	0.0100	0.77a	0.0100	0.75bc	0.0067	0.74ab	0.0100
	Treated Shade	0.82a	0.0000	0.81ac	0.0000	0.79b	0.0000	0.77a	0.0033	0.76c	0.0033	0.74b	0.0033

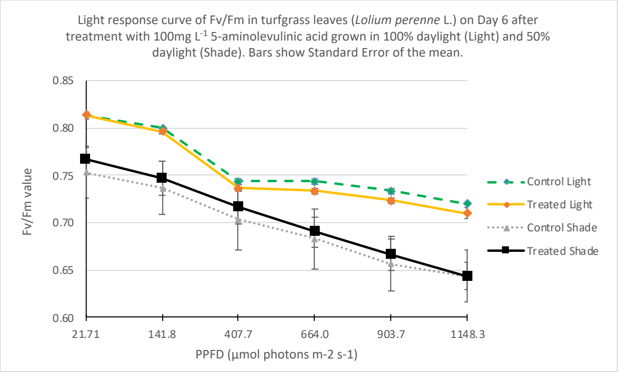
Light response curves for Maximum Quantum Efficiency of PSII Photochemistry (F_v/F_m)

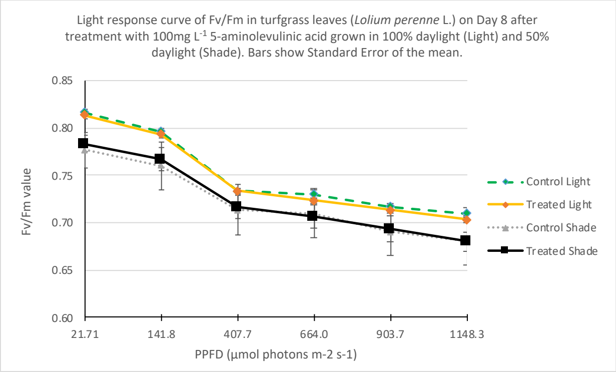
Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)

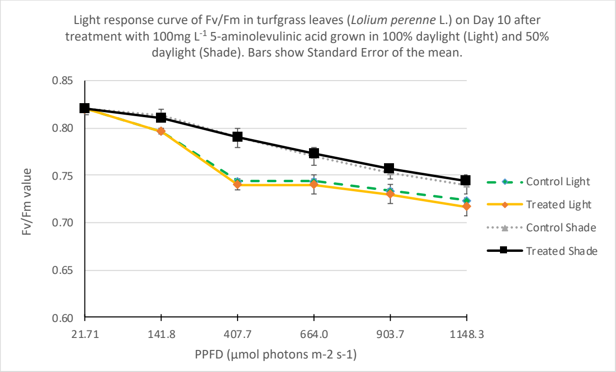

Light response curve of Fv/Fm in turfgrass leaves (*Lolium perenne* L.) on Day 0 (four hours) after treatment with 100mg L^{-1} 5-aminolevulinic acid grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.


Light response curve of Fv/Fm in turfgrass leaves (*Lolium perenne* L.) on Day 2 after treatment with 100mg L^{-1} 5-aminolevulinic acid grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.


Light response curve of Fv/Fm in turfgrass leaves (*Lolium perenne* L.) on Day 4 after treatment with 100mg L^{-1} 5-aminolevulinic acid grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.

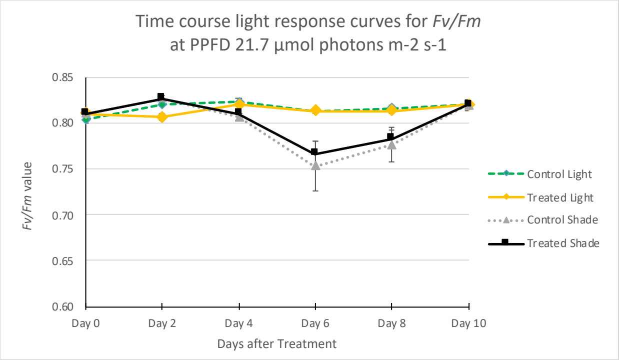

Light response curve of Fv/Fm in turfgrass leaves (*Lolium perenne* L.) on Day 6 after treatment with 100mg L^{-1} 5-aminolevulinic acid grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.

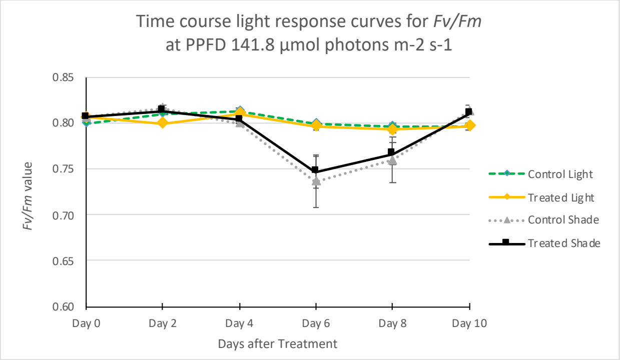

Light response curve of Fv/Fm in turfgrass leaves (*Lolium perenne* L.) on Day 8 after treatment with 100mg L^{-1} 5-aminolevulinic acid grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.

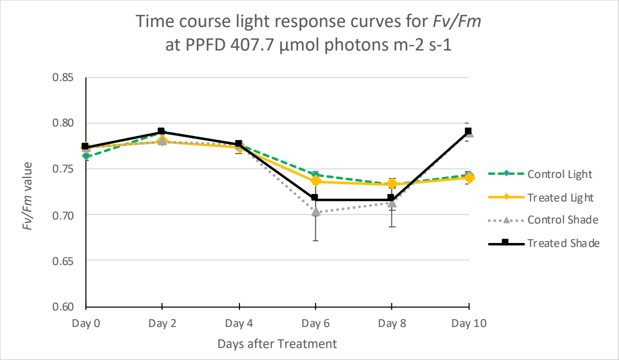

Light response curve of Fv/Fm in turfgrass leaves (*Lolium perenne* L.) on Day 10 after treatment with 100mg L^{-1} 5-aminolevulinic acid grown in 100% daylight (Light) and 50% daylight (Shade). Bars show Standard Error of the mean.

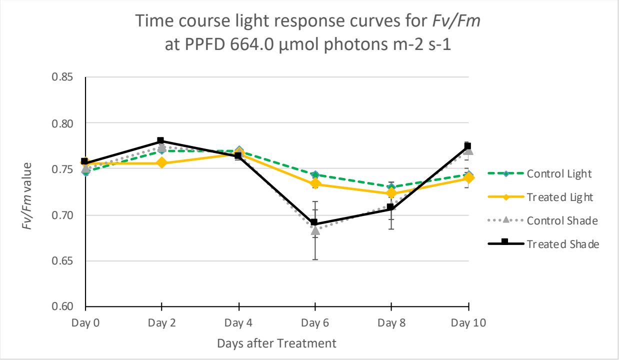
Time course response curves for Maximum Quantum Efficiency of PSII Photochemistry (F_v/F_m)

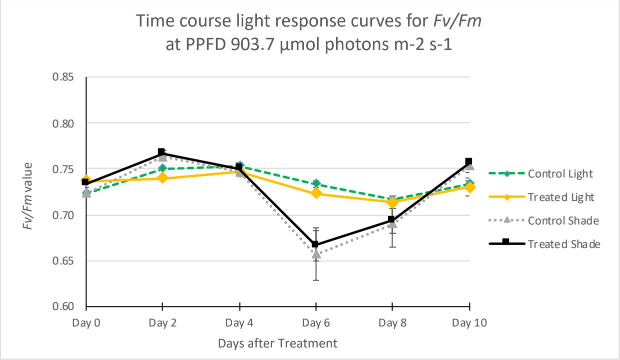
Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)

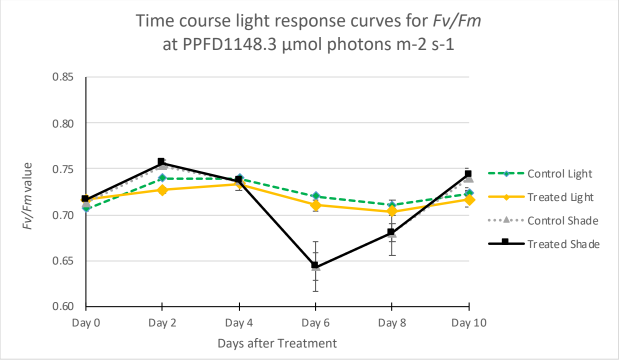

Time course light response curves for *Fv/Fm* at PPFD 21.7 µmol photons m-2 s-1


Time course light response curves for *Fv/Fm* at PPFD 141.8 µmol photons m-2 s-1


Time course light response curves for *Fv/Fm* at PPFD 407.7 µmol photons m-2 s-1


Time course light response curves for *Fv/Fm* at PPFD 664.0 µmol photons m-2 s-1


Time course light response curves for *Fv/Fm* at PPFD 903.7 µmol photons m-2 s-1


Time course light response curves for *Fv/Fm* at PPFD1148.3 µmol photons m-2 s-1

ANOVA for Fv/Fm

DAY 0

Variate: Fv/Fm_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	1.00	0.347
Light	1	0.00003333	0.00003333	1.00	0.347
ALA.Light	1	0.00003333	0.00003333	1.00	0.347
Residual	8	0.00026667	0.00003333		
Total	11	0.00036667			

Variate: Fv/Fm_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	1.33	0.282
Light	1	0.00003333	0.00003333	1.33	0.282
ALA.Light	1	0.00003333	0.00003333	1.33	0.282
Residual	8	0.00020000	0.00002500		
Total	11	0.00030000			

Variate: Fv/Fm_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00007500	0.00007500	2.25	0.172
Light	1	0.00007500	0.00007500	2.25	0.172
ALA.Light	1	0.00007500	0.00007500	2.25	0.172
Residual	8	0.00026667	0.00003333		
Total	11	0.00049167			

Variate: Fv/Fm_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00020833	0.00020833	8.33	0.020
Light	1	0.00000833	0.00000833	0.33	0.580
ALA.Light	1	0.00000833	0.00000833	0.33	0.580
Residual	8	0.00020000	0.00002500		
Total	11	0.00042500			

Variate: Fv/Fm_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00040833	0.00040833	12.25	0.008
Light	1	0.00000833	0.00000833	0.25	0.631
ALA.Light	1	0.00000833	0.00000833	0.25	0.631
Residual	8	0.00026667	0.00003333		
Total	11	0.00069167			

Variate: Fv/Fm_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00013333	0.00013333	4.00	0.081
Light	1	0.00003333	0.00003333	1.00	0.347
ALA.Light	1	0.00003333	0.00003333	1.00	0.347
Residual	8	0.00026667	0.00003333		
Total	11	0.00046667			

DAY 2

Variate: Fv/Fm_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00013333	0.00013333	5.33	0.050
Light	1	0.00053333	0.00053333	21.33	0.002
ALA.Light	1	0.00013333	0.00013333	5.33	0.050
Residual	8	0.00020000	0.00002500		
Total	11	0.00100000			

Variate: Fv/Fm_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00013333	0.00013333	8.00	0.022
Light	1	0.00030000	0.00030000	18.00	0.003
ALA.Light	1	0.00003333	0.00003333	2.00	0.195
Residual	8	0.00013333	0.00001667		
Total	11	0.00060000			

Variate: Fv/Fm_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	8.333E-08	8.333E-08	1.00	0.347
Light	1	8.333E-08	8.333E-08	1.00	0.347
ALA.Light	1	2.901E-04	2.901E-04	3481.00	<.001
Residual	8	6.667E-07	8.333E-08		
Total	11	2.909E-04			

Variate: Fv/Fm_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	2.00	0.195
Light	1	0.00053333	0.00053333	32.00	<.001
ALA.Light	1	0.00030000	0.00030000	18.00	0.003
Residual	8	0.00013333	0.00001667		
Total	11	0.00100000			

Variate: Fv/Fm_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	2.00	0.195
Light	1	0.00120000	0.00120000	72.00	<.001
ALA.Light	1	0.00013333	0.00013333	8.00	0.022
Residual	8	0.00013333	0.00001667		
Total	11	0.00150000			

Variate: Fv/Fm_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00007500	0.00007500	3.00	0.122
Light	1	0.00140833	0.00140833	56.33	<.001
ALA.Light	1	0.00020833	0.00020833	8.33	0.020
Residual	8	0.00020000	0.00002500		
Total	11	0.00189167			

DAY 4

Variate: Fv/Fm_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000000	0.00000000	0.00	1.000
Light	1	0.00053333	0.00053333	32.00	<.001
ALA.Light	1	0.00003333	0.00003333	2.00	0.195
Residual	8	0.00013333	0.00001667		
Total	11	0.00070000			

Variate: Fv/Fm_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000000	0.00000000	0.00	1.000
Light	1	0.00030000	0.00030000	18.00	0.003
ALA.Light	1	0.00003333	0.00003333	2.00	0.195
Residual	8	0.00013333	0.00001667		
Total	11	0.00046667			

Variate: Fv/Fm_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000833	0.00000833	0.14	0.715
Light	1	0.00000833	0.00000833	0.14	0.715
ALA.Light	1	0.00000833	0.00000833	0.14	0.715
Residual	8	0.00046667	0.00005833		
Total	11	0.00049167			

Variate: Fv/Fm_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	1.33	0.282
Light	1	0.00003333	0.00003333	1.33	0.282
ALA.Light	1	0.00000000	0.00000000	0.00	1.000
Residual	8	0.00020000	0.00002500		
Total	11	0.00026667			

Variate: Fv/Fm_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000833	0.00000833	0.17	0.694
Light	1	0.00000833	0.00000833	0.17	0.694
ALA.Light	1	0.00007500	0.00007500	1.50	0.256
Residual	8	0.00040000	0.00005000		
Total	11	0.00049167			

Variate: Fv/Fm_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	0.67	0.438
Light	1	0.00000000	0.00000000	0.00	1.000
ALA.Light	1	0.00003333	0.00003333	0.67	0.438
Residual	8	0.00040000	0.00005000		
Total	11	0.00046667			

DAY 6

Variate: Fv/Fm_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0001333	0.0001333	0.19	0.676
Light	1	0.0085333	0.0085333	12.05	0.008
ALA.Light	1	0.0001333	0.0001333	0.19	0.676
Residual	8	0.0056667	0.0007083		
Total	11	0.0144667			

Variate: Fv/Fm_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.04	0.848
Light	1	0.0096333	0.0096333	11.33	0.010
ALA.Light	1	0.0001333	0.0001333	0.16	0.702
Residual	8	0.0068000	0.0008500		
Total	11	0.0166000			

Variate: Fv/Fm_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000033	0.000033	0.03	0.860
Light	1	0.002700	0.002700	2.68	0.140
ALA.Light	1	0.000300	0.000300	0.30	0.600
Residual	8	0.008067	0.001008		
Total	11	0.011100			

Variate: Fv/Fm_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.01	0.928
Light	1	0.0080083	0.0080083	8.43	0.020
ALA.Light	1	0.0002083	0.0002083	0.22	0.652
Residual	8	0.0076000	0.0009500		
Total	11	0.0158250			

Variate: Fv/Fm_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000000	0.0000000	0.00	1.000
Light	1	0.0133333	0.0133333	16.00	0.004
ALA.Light	1	0.0003000	0.0003000	0.36	0.565
Residual	8	0.0066667	0.0008333		
Total	11	0.0203000			

Variate: Fv/Fm_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.10	0.759
Light	1	0.0154083	0.0154083	20.78	0.002
ALA.Light	1	0.0000750	0.0000750	0.10	0.759
Residual	8	0.0059333	0.0007417		
Total	11	0.0214917			

DAY 8

Variate: Fv/Fm_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.03	0.878
Light	1	0.0036750	0.0036750	11.02	0.011
ALA.Light	1	0.0000750	0.0000750	0.22	0.648
Residual	8	0.0026667	0.0003333		
Total	11	0.0064250			

Variate: Fv/Fm_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.01	0.909
Light	1	0.0030083	0.0030083	5.01	0.055
ALA.Light	1	0.0000750	0.0000750	0.13	0.733
Residual	8	0.0048000	0.0006000		
Total	11	0.0078917			

Variate: Fv/Fm_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.01	0.913
Light	1	0.0010083	0.0010083	1.53	0.251
ALA.Light	1	0.0000083	0.0000083	0.01	0.913
Residual	8	0.0052667	0.0006583		
Total	11	0.0062917			

Variate: Fv/Fm_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.12	0.736
Light	1	0.0010083	0.0010083	1.64	0.237
ALA.Light	1	0.0000083	0.0000083	0.01	0.910
Residual	8	0.0049333	0.0006167		
Total	11	0.0060250			

Variate: Fv/Fm_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000000	0.0000000	0.00	1.000
Light	1	0.0016333	0.0016333	2.61	0.145
ALA.Light	1	0.0000333	0.0000333	0.05	0.823
Residual	8	0.0050000	0.0006250		
Total	11	0.0066667			

Variate: Fv/Fm_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.06	0.817
Light	1	0.0021333	0.0021333	3.66	0.092
ALA.Light	1	0.0000333	0.0000333	0.06	0.817
Residual	8	0.0046667	0.0005833		
Total	11	0.0068667			

DAY 10

Variate: Fv/Fm_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000000	0.00000000	0.00	1.000
Light	1	0.00000000	0.00000000	0.00	1.000
ALA.Light	1	0.00000000	0.00000000	0.00	1.000
Residual	8	0.00020000	0.00002500		
Total	11	0.00020000			

Variate: Fv/Fm_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000833	0.00000833	0.17	0.694
Light	1	0.00067500	0.00067500	13.50	0.006
ALA.Light	1	0.00000833	0.00000833	0.17	0.694
Residual	8	0.00040000	0.00005000		
Total	11	0.00109167			

Variate: Fv/Fm_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.08	0.789
Light	1	0.0070083	0.0070083	64.69	<.001
ALA.Light	1	0.0000083	0.0000083	0.08	0.789
Residual	8	0.0008667	0.0001083		
Total	11	0.0078917			

Variate: Fv/Fm _Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000000	0.0000000	0.00	1.000
Light	1	0.0027000	0.0027000	16.20	0.004
ALA.Light	1	0.0000333	0.0000333	0.20	0.667
Residual	8	0.0013333	0.0001667		
Total	11	0.0040667			

Variate: Fv/Fm _Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000000	0.0000000	0.00	1.000
Light	1	0.0016333	0.0016333	13.07	0.007
ALA.Light	1	0.0000333	0.0000333	0.27	0.620
Residual	8	0.0010000	0.0001250		
Total	11	0.0026667			

Variate: Fv/Fm_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.06	0.820
Light	1	0.0014083	0.0014083	9.39	0.015
ALA.Light	1	0.0000750	0.0000750	0.50	0.500
Residual	8	0.0012000	0.0001500		
Total	11	0.0026917			

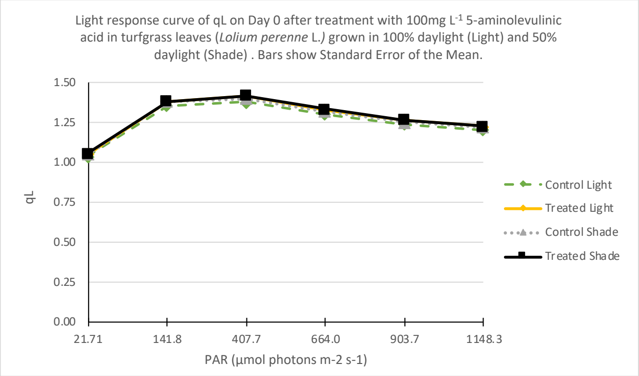
qL – (Fraction of open PSII reaction centres, lake model)

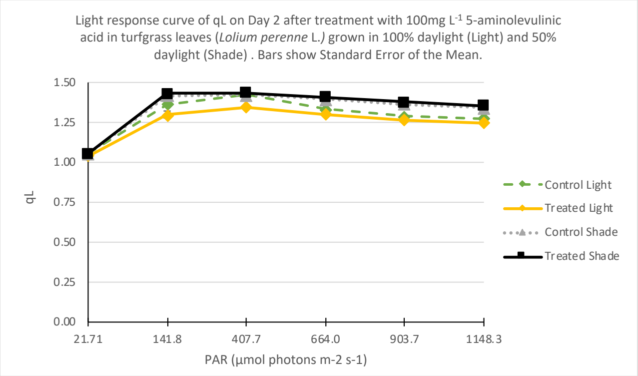
Means with same letters within datasets Days After Treatment and PAR level are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

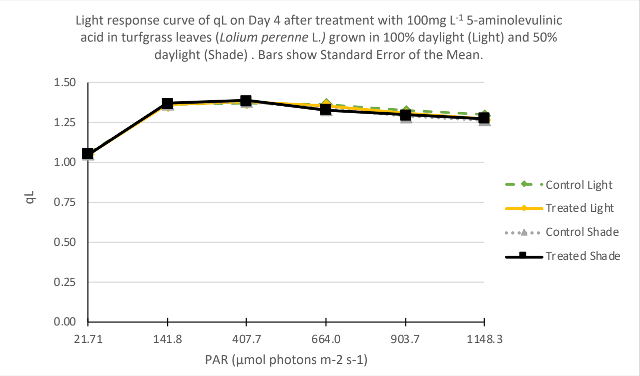
	% of Full Daylight	**10.00%**		**20.00%**		**40.00%**		**60.00%**		**80.00%**		**100.00%**	
	PAR (µmol photons m-2s-1)	**21.71**		**141.80**		**407.70**		**664.00**		**903.70**		**1148.30**	
Days after treatment	**Treated/light**	**Mean qL**	**St. Error**	**Mean qL**	**St. Error**	**Mean qL**	**St. Error**	**Mean qL**	**St. Error**	**Mean qL**	**St. Error**	**Mean qL**	**St. Error**
Day 0	Control Light	1.04a	±0.0033	1.35a	±0.0088	1.37a	±0.0145	1.30a	±0.0088	1.24b	±0.0033	1.20a	±0.0058
	Treated Light	1.05b	±0.0033	1.38a	±0.0145	1.41a	±0.0173	1.33a	±0.0145	1.26a	±0.0120	1.22b	±0.0067
	Control Shade	1.05b	±0.0000	1.38a	±0.0200	1.40a	±0.0120	1.32a	±0.0120	1.25a	±0.0100	1.22ab	±0.0067
	Treated Shade	1.05b	±0.0033	1.38a	±0.0033	1.41a	±0.0067	1.33a	±0.0058	1.26a	±0.0033	1.22b	±0.0033
Day 2	Control Light	1.05a	±0.0067	1.36b	±0.0000	1.42b	±0.0058	1.33b	±0.0058	1.29b	±0.0033	1.27b	0.0058
	Treated Light	1.04a	±0.0000	1.29a	±0.0203	1.34a	±0.0088	1.30a	±0.0033	1.26a	±0.0058	1.24a	0.0033
	Control Shade	1.05a	±0.0033	1.41c	±0.0260	1.42b	±0.0100	1.39c	±0.0088	1.36c	±0.0100	1.34c	0.0120
	Treated Shade	1.05a	±0.0067	1.43bc	±0.0203	1.43b	±0.0067	1.41c	±0.0088	1.37c	±0.0088	1.35c	0.0088
Day 4	Control Light	1.05a	±0.0067	1.36a	±0.0115	1.37a	±0.0000	1.36a	±0.0000	1.32a	±0.0058	1.29a	±0.0033
	Treated Light	1.05a	±0.0067	1.36a	±0.0120	1.37a	±0.0167	1.35a	±0.0100	1.30a	±0.0120	1.27a	±0.0133
	Control Shade	1.05a	±0.0033	1.37a	±0.0186	1.38a	±0.0115	1.33a	±0.0153	1.29a	±0.0145	1.26a	±0.0120
	Treated Shade	1.05a	±0.0058	1.36a	±0.0145	1.38a	±0.0133	1.33a	±0.0120	1.29a	±0.0088	1.27a	±0.0088
Day 6	Control Light	1.06a	±0.0033	1.36ab	±0.0033	1.31a	±0.0100	1.30a	±0.0058	1.28b	±0.0058	1.25b	±0.0033
	Treated Light	1.05a	±0.0033	1.37b	±0.0067	1.28a	±0.0033	1.27a	±0.0067	1.24ab	±0.0120	1.20ab	±0.0176
	Control Shade	1.05a	±0.0000	1.25a	±0.0503	1.19a	±0.0835	1.12a	±0.1002	1.04a	±0.1186	0.98a	±0.1239
	Treated Shade	1.05a	±0.0033	1.27ab	±0.0433	1.24a	±0.0441	1.16a	±0.0384	1.09ab	±0.0416	1.01a	±0.0436
Day 8	Control Light	1.05b	±0.0067	1.38a	±0.0058	1.28a	±0.0088	1.26a	±0.0115	1.23a	±0.0115	1.20a	±0.0145
	Treated Light	1.05ab	±0.0058	1.37a	±0.0033	1.28a	±0.0100	1.26a	±0.0120	1.22a	±0.0133	1.19a	±0.0133
	Control Shade	1.04ab	±0.0033	1.30a	±0.0546	1.23a	±0.0600	1.21a	±0.0513	1.18a	±0.0513	1.15a	±0.0458
	Treated Shade	1.03a	±0.0033	1.32a	±0.0416	1.23a	±0.0384	1.21a	±0.0318	1.18a	±0.0267	1.15a	±0.0252
Day 10	Control Light	1.06b	±0.0033	1.37a	±0.0088	1.30a	±0.0067	1.31a	±0.0133	1.27a	±0.0145	1.23a	±0.0176
	Treated Light	1.05a	±0.0033	1.37a	±0.0000	1.29a	±0.0133	1.29a	±0.0300	1.25a	±0.0418	1.21a	±0.0484
	Control Shade	1.05a	±0.0067	1.41b	±0.0088	1.43b	±0.0318	1.36b	±0.0252	1.30a	±0.0219	1.28a	±0.0186
	Treated Shade	1.05a	±0.0067	1.42b	±0.0058	1.45b	±0.0058	1.36b	±0.0088	1.31a	±0.0067	1.29a	±0.0067

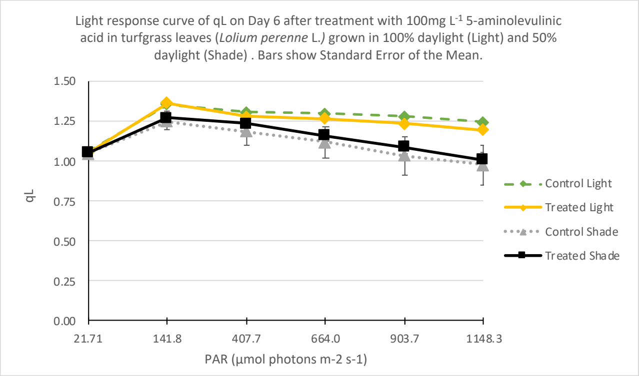
qL – (Fraction of open PSII reaction centres, lake model)

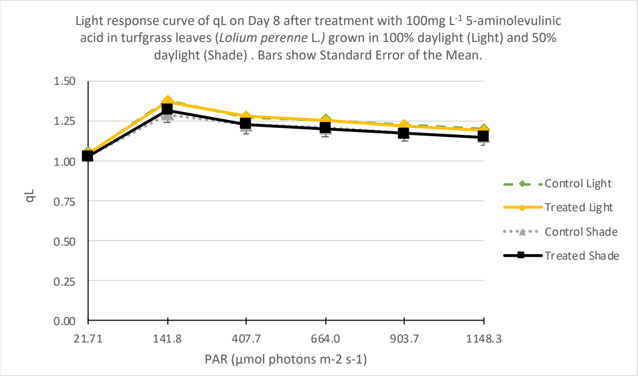
Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)

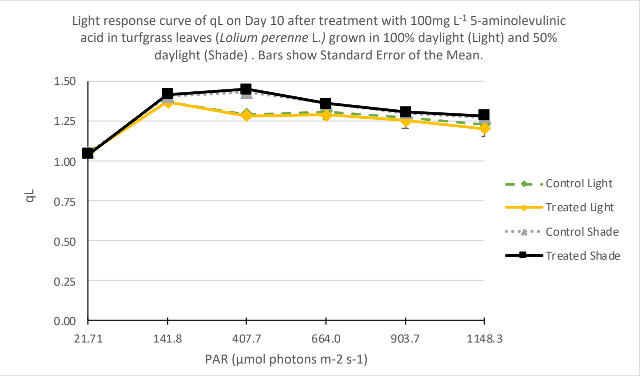

Light response curve of qL on Day 0 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.


Light response curve of qL on Day 2 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.


Light response curve of qL on Day 4 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

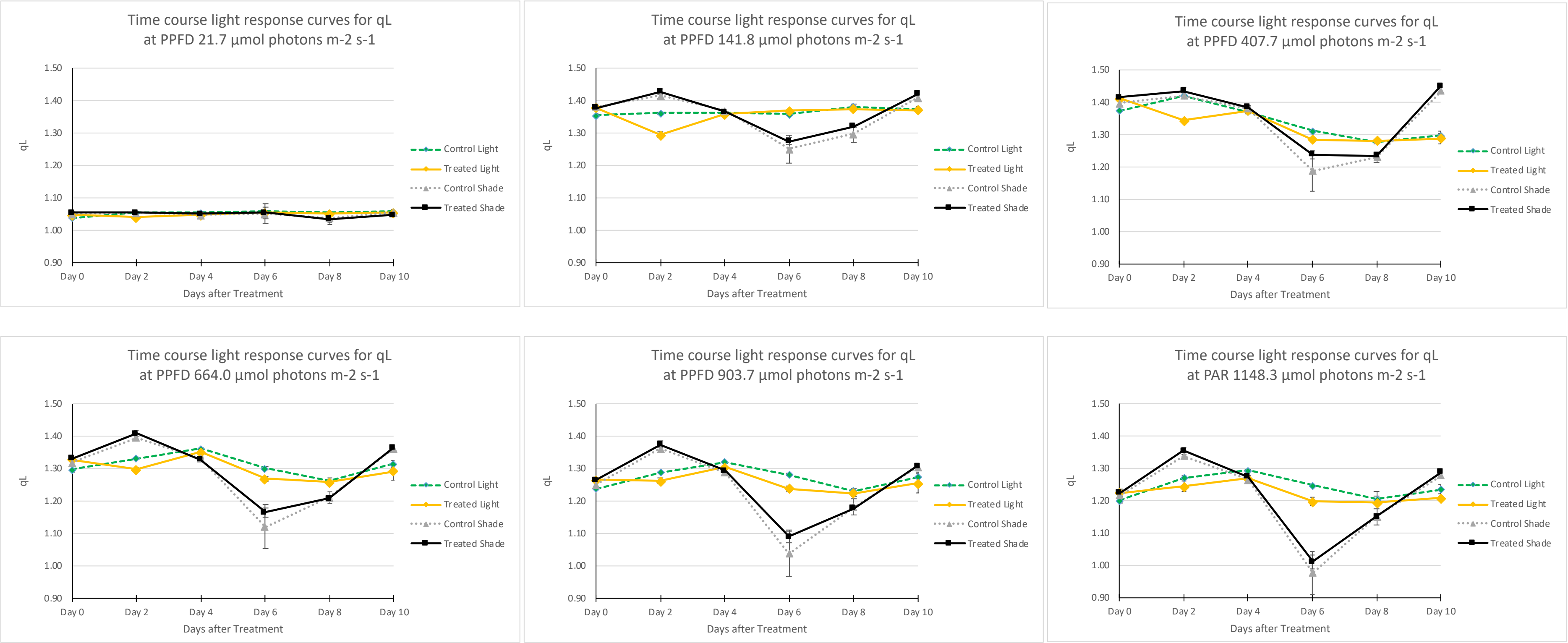

Light response curve of qL on Day 6 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.


Light response curve of qL on Day 8 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.


Light response curve of qL on Day 10 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

Time course response curves for qL – (Fraction of open PSII reaction centres, lake model)

Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)



ANOVA for qL

DAY 0

Variate: qL_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00013333	0.00013333	5.33	0.050
Light	1	0.00030000	0.00030000	12.00	0.009
ALA.Light	1	0.00003333	0.00003333	1.33	0.282
Residual	8	0.00020000	0.00002500		
Total	11	0.00066667			

Variate: qL _Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0003000	0.0003000	0.57	0.471
Light	1	0.0005333	0.0005333	1.02	0.343
ALA.Light	1	0.0005333	0.0005333	1.02	0.343
Residual	8	0.0042000	0.0005250		
Total	11	0.0055667			

Variate: qL _Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0021333	0.0021333	4.06	0.079
Light	1	0.0005333	0.0005333	1.02	0.343
ALA.Light	1	0.0003000	0.0003000	0.57	0.471
Residual	8	0.0042000	0.0005250		
Total	11	0.0071667			

Variate: qL _Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0014083	0.0014083	4.02	0.080
Light	1	0.0004083	0.0004083	1.17	0.312
ALA.Light	1	0.0002083	0.0002083	0.60	0.463
Residual	8	0.0028000	0.0003500		
Total	11	0.0048250			

Variate: qL _Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0012000	0.0012000	6.00	0.040
Light	1	0.0001333	0.0001333	0.67	0.438
ALA.Light	1	0.0001333	0.0001333	0.67	0.438
Residual	8	0.0016000	0.0002000		
Total	11	0.0030667			

Variate: qL _Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0006750	0.0006750	6.75	0.032
Light	1	0.0002083	0.0002083	2.08	0.187
ALA.Light	1	0.0002083	0.0002083	2.08	0.187
Residual	8	0.0008000	0.0001000		
Total	11	0.0018917			

DAY 2

Variate: qL_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00013333	0.00013333	1.78	0.219
Light	1	0.00013333	0.00013333	1.78	0.219
ALA.Light	1	0.00013333	0.00013333	1.78	0.219
Residual	8	0.00060000	0.00007500		
Total	11	0.00100000			

Variate: qL _Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.002133	0.002133	1.90	0.206
Light	1	0.026133	0.026133	23.23	0.001
ALA.Light	1	0.004800	0.004800	4.27	0.073
Residual	8	0.009000	0.001125		
Total	11	0.042067			

Variate: qL _Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0030083	0.0030083	15.70	0.004
Light	1	0.0060750	0.0060750	31.70	<.001
ALA.Light	1	0.0060750	0.0060750	31.70	<.001
Residual	8	0.0015333	0.0001917		
Total	11	0.0166917			

Variate: qL _Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0003000	0.0003000	2.00	0.195
Light	1	0.0225333	0.0225333	150.22	<.001
ALA.Light	1	0.0016333	0.0016333	10.89	0.011
Residual	8	0.0012000	0.0001500		
Total	11	0.0256667			

Variate: qL _Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0001333	0.0001333	0.80	0.397
Light	1	0.0261333	0.0261333	156.80	<.001
ALA.Light	1	0.0012000	0.0012000	7.20	0.028
Residual	8	0.0013333	0.0001667		
Total	11	0.0288000			

Variate: qL _Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.37	0.557
Light	1	0.0234083	0.0234083	117.04	<.001
ALA.Light	1	0.0014083	0.0014083	7.04	0.029
Residual	8	0.0016000	0.0002000		
Total	11	0.0264917			

DAY 4

Variate: qL _Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.08	0.780
Light	1	0.0000083	0.0000083	0.08	0.780
ALA.Light	1	0.0000750	0.0000750	0.75	0.412
Residual	8	0.0008000	0.0001000		
Total	11	0.0008917			

Variate: qL _Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.05	0.823
Light	1	0.0001333	0.0001333	0.21	0.656
ALA.Light	1	0.0000000	0.0000000	0.00	1.000
Residual	8	0.0050000	0.0006250		
Total	11	0.0051667			

Variate: qL _Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.08	0.790
Light	1	0.0003000	0.0003000	0.68	0.434
ALA.Light	1	0.0000000	0.0000000	0.00	1.000
Residual	8	0.0035333	0.0004417		
Total	11	0.0038667			

Variate: qL _Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0001333	0.0001333	0.37	0.559
Light	1	0.0021333	0.0021333	5.95	0.041
ALA.Light	1	0.0000333	0.0000333	0.09	0.768
Residual	8	0.0028667	0.0003583		
Total	11	0.0051667			

Variate: qL _Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.21	0.656
Light	1	0.0014083	0.0014083	4.02	0.080
ALA.Light	1	0.0004083	0.0004083	1.17	0.312
Residual	8	0.0028000	0.0003500		
Total	11	0.0046917			

Variate: qL _Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0002083	0.0002083	0.68	0.435
Light	1	0.0004083	0.0004083	1.32	0.283
ALA.Light	1	0.0010083	0.0010083	3.27	0.108
Residual	8	0.0024667	0.0003083		
Total	11	0.0040917			

DAY 6

Variate: qL _Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000000	0.00000000	0.00	1.000
Light	1	0.00003333	0.00003333	1.33	0.282
ALA.Light	1	0.00003333	0.00003333	1.33	0.282
Residual	8	0.00020000	0.00002500		
Total	11	0.00026667			

Variate: qL _Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000833	0.000833	0.25	0.631
Light	1	0.030000	0.030000	8.96	0.017
ALA.Light	1	0.000133	0.000133	0.04	0.847
Residual	8	0.026800	0.003350		
Total	11	0.057767			

Variate: qL_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000408	0.000408	0.06	0.812
Light	1	0.021675	0.021675	3.20	0.111
ALA.Light	1	0.004408	0.004408	0.65	0.443
Residual	8	0.054200	0.006775		
Total	11	0.080692			

Variate: qL _Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000075	0.000075	0.01	0.928
Light	1	0.060208	0.060208	6.93	0.030
ALA.Light	1	0.004408	0.004408	0.51	0.497
Residual	8	0.069533	0.008692		
Total	11	0.134225			

Variate: qL _Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00007	0.00007	0.01	0.939
Light	1	0.11407	0.11407	9.51	0.015
ALA.Light	1	0.00701	0.00701	0.58	0.467
Residual	8	0.09593	0.01199		
Total	11	0.21709			

Variate: qL _Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00021	0.00021	0.02	0.903
Light	1	0.15641	0.15641	11.87	0.009
ALA.Light	1	0.00521	0.00521	0.40	0.547
Residual	8	0.10540	0.01318		
Total	11	0.26723			

DAY 8

Variate: qL _Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	0.44	0.524
Light	1	0.00083333	0.00083333	11.11	0.010
ALA.Light	1	0.00000000	0.00000000	0.00	1.000
Residual	8	0.00060000	0.00007500		
Total	11	0.00146667			

Variate: qL _Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000208	0.000208	0.06	0.815
Light	1	0.014008	0.014008	3.93	0.083
ALA.Light	1	0.000675	0.000675	0.19	0.675
Residual	8	0.028533	0.003567		
Total	11	0.043425			

Variate: qL _Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000033	0.000033	0.01	0.929
Light	1	0.006533	0.006533	1.66	0.234
ALA.Light	1	0.000000	0.000000	0.00	1.000
Residual	8	0.031533	0.003942		
Total	11	0.038100			

Variate: qL _Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000033	0.000033	0.01	0.918
Light	1	0.007500	0.007500	2.55	0.149
ALA.Light	1	0.000000	0.000000	0.00	1.000
Residual	8	0.023533	0.002942		
Total	11	0.031067			

Variate: qL _Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000075	0.000075	0.03	0.873
Light	1	0.007008	0.007008	2.56	0.149
ALA.Light	1	0.000008	0.000008	0.00	0.957
Residual	8	0.021933	0.002742		
Total	11	0.029025			

Variate: qL _Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000075	0.000075	0.03	0.862
Light	1	0.007008	0.007008	2.99	0.122
ALA.Light	1	0.000075	0.000075	0.03	0.862
Residual	8	0.018733	0.002342		
Total	11	0.025892			

DAY 10

Variate: qL _Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00007500	0.00007500	0.90	0.371
Light	1	0.00007500	0.00007500	0.90	0.371
ALA.Light	1	0.00000833	0.00000833	0.10	0.760
Residual	8	0.00066667	0.00008333		
Total	11	0.00082500			

Variate: qL _Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.53	0.488
Light	1	0.0052083	0.0052083	36.76	<.001
ALA.Light	1	0.0002083	0.0002083	1.47	0.260
Residual	8	0.0011333	0.0001417		
Total	11	0.0066250			

Variate: qL _Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.04	0.856
Light	1	0.0675000	0.0675000	71.05	<.001
ALA.Light	1	0.0005333	0.0005333	0.56	0.475
Residual	8	0.0076000	0.0009500		
Total	11	0.0756667			

Variate: qL _Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000300	0.000300	0.22	0.649
Light	1	0.010800	0.010800	8.05	0.022
ALA.Light	1	0.000533	0.000533	0.40	0.546
Residual	8	0.010733	0.001342		
Total	11	0.022367			

Variate: qL _Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000208	0.000208	0.11	0.746
Light	1	0.005208	0.005208	2.80	0.133
ALA.Light	1	0.000408	0.000408	0.22	0.652
Residual	8	0.014867	0.001858		
Total	11	0.020692			

Variate: qL _Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000208	0.000208	0.09	0.770
Light	1	0.011408	0.011408	5.00	0.056
ALA.Light	1	0.001008	0.001008	0.44	0.525
Residual	8	0.018267	0.002283		
Total	11	0.030892			

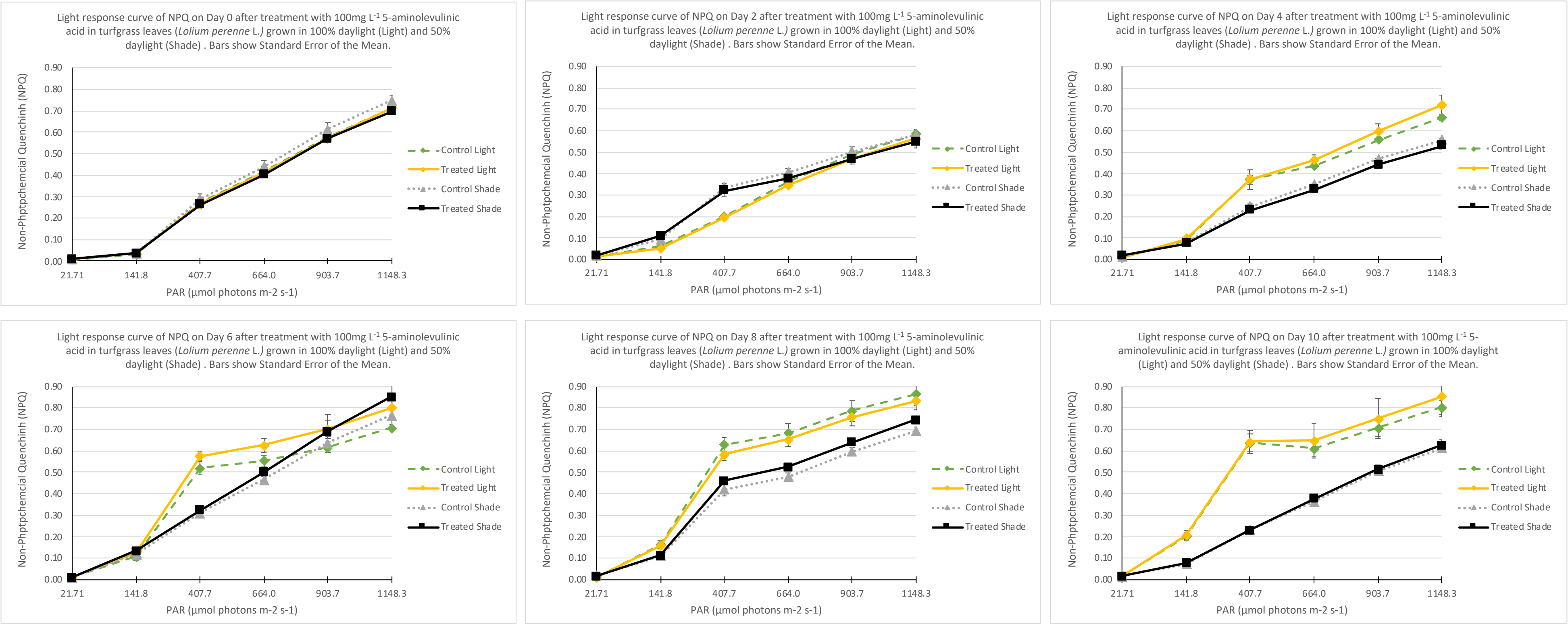
NPQ (Non-Photochemical Quenching)

Means with same letters within datasets Days After Treatment and PAR level are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

	% of Full Daylight	10.00%		20.00%		40.00%		60.00%		80.00%		100.00%	
	PAR (µmol photons m-2s-1)	21.71		141.80		407.70		664.00		903.70		1148.30	
Days after treament	Treated/light	Mean ØPSII	St. Error	Mean ØPSII	St. Error	Mean ØPSII	St. Error	Mean ØPSII	St. Error	Mean ØPSII	St. Error	Mean ØPSII	St. Error
Day 0	Control Light	0.01a	±0.0033	0.03a	±0.0058	0.27a	±0.0240	0.42a	±0.0233	0.58a	±0.0240	0.71a	±0.0318
	Treated Light	0.01a	±0.0000	0.04a	±0.0033	0.27a	±0.0088	0.41a	±0.0088	0.57a	±0.0153	0.71a	±0.0186
	Control Shade	0.01a	±0.0000	0.04a	±0.0033	0.29a	±0.0231	0.44a	±0.0265	0.61a	±0.0296	0.75a	±0.0273
	Treated Shade	0.01a	±0.0000	0.04a	±0.0033	0.26a	±0.0219	0.40a	±0.0120	0.57a	±0.0115	0.70a	±0.0088
Day 2	Control Light	0.01a	±0.0033	0.06a	±0.0000	0.20a	±0.0058	0.36ab	±0.0058	0.49a	±0.0058	0.58a	±0.0033
	Treated Light	0.01a	±0.0033	0.05a	±0.0058	0.19a	±0.0033	0.34a	±0.0033	0.47a	±0.0100	0.56a	±0.0176
	Control Shade	0.02a	±0.0033	0.09b	±0.0088	0.34b	±0.0176	0.41b	±0.0145	0.50a	±0.0203	0.58a	±0.0265
	Treated Shade	0.02a	±0.0033	0.11b	±0.0153	0.32b	±0.0231	0.38ab	±0.0233	0.47a	±0.0240	0.55a	±0.0296
Day 4	Control Light	0.01a	±0.0000	0.09a	±0.0033	0.37b	±0.0200	0.44b	±0.0088	0.56b	0.0067	0.66b	±0.0100
	Treated Light	0.01a	±0.0000	0.10a	±0.0120	0.37b	±0.0437	0.46b	±0.0240	0.60b	0.0367	0.72b	±0.0451
	Control Shade	0.01a	±0.0033	0.08a	±0.0058	0.25a	±0.0145	0.35a	±0.0058	0.47a	0.0033	0.56a	±0.0067
	Treated Shade	0.02a	±0.0033	0.07a	±0.0033	0.23a	±0.0058	0.33a	±0.0145	0.44a	0.0153	0.53a	±0.0153
Day 6	Control Light	0.01a	±0.0000	0.11a	±0.0176	0.52b	±0.0306	0.55ab	±0.0260	0.62a	±0.0219	0.71a	±0.0120
	Treated Light	0.01a	±0.0000	0.13a	±0.0067	0.58b	±0.0219	0.63b	±0.0328	0.70a	±0.0436	0.80a	±0.0462
	Control Shade	0.01a	±0.0000	0.12a	±0.0145	0.31a	±0.0208	0.47a	±0.0145	0.64a	±0.0318	0.77a	±0.0441
	Treated Shade	0.01a	±0.0000	0.13a	±0.0067	0.32a	±0.0145	0.50a	±0.0436	0.69a	±0.0794	0.85a	±0.1058
Day 8	Control Light	0.01ab	±0.0033	0.16b	±0.0176	0.63b	±0.0300	0.68b	±0.0437	0.79b	±0.0470	0.86b	±0.0536
	Treated Light	0.01a	±0.0000	0.16b	±0.0145	0.58b	±0.0291	0.65b	±0.0328	0.76b	±0.0393	0.83b	±0.0441
	Control Shade	0.02b	±0.0000	0.11a	±0.0208	0.42a	±0.0289	0.48a	±0.0173	0.60a	±0.0186	0.69a	±0.0176
	Treated Shade	0.02ab	±0.0033	0.11a	±0.0033	0.46a	±0.0153	0.52a	±0.0088	0.64a	±0.0173	0.74ab	±0.0145
Day 10	Control Light	0.02a	±0.0000	0.20b	±0.0208	0.64b	±0.0410	0.61b	±0.0404	0.71b	±0.0384	0.80b	±0.0318
	Treated Light	0.02a	±0.0000	0.21b	±0.0203	0.64b	±0.0536	0.65b	±0.0817	0.75b	±0.0954	0.85b	±0.0940
	Control Shade	0.02a	±0.0033	0.07a	±0.0033	0.23a	±0.0033	0.36a	±0.0088	0.51a	±0.0067	0.61a	±0.0033
	Treated Shade	0.02a	±0.0033	0.08a	±0.0033	0.23a	±0.0058	0.38a	±0.0145	0.51a	±0.0203	0.63a	±0.0233

Light response curves for NPQ (Non-Photochemical Quenching)

Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)



Light response curve of NPQ on Day 0 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

Light response curve of NPQ on Day 2 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

Light response curve of NPQ on Day 4 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

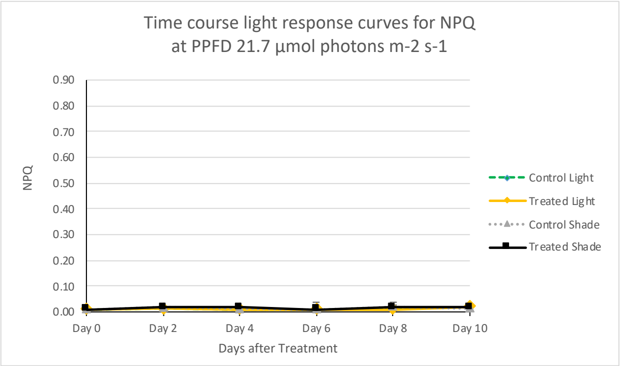
Light response curve of NPQ on Day 6 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

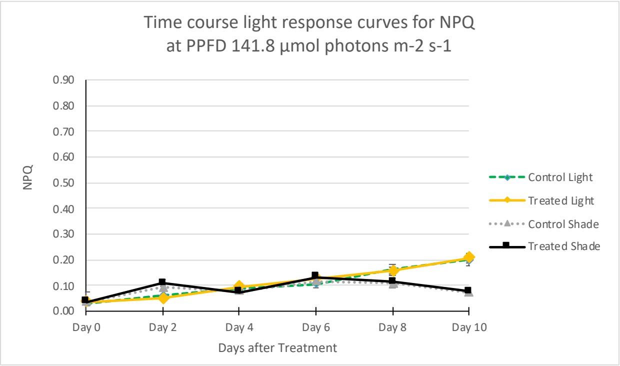
Light response curve of NPQ on Day 8 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

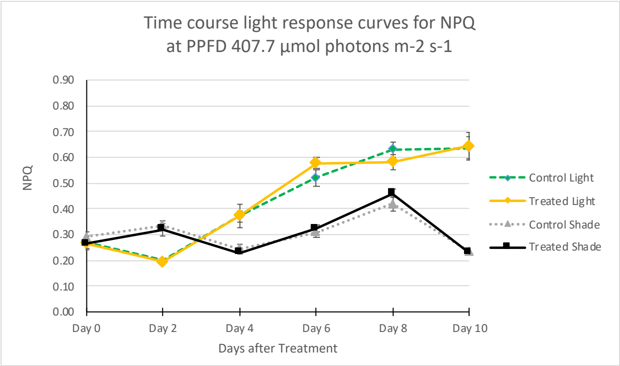
Light response curve of NPQ on Day 10 after treatment with 100mg L^{-1} 5-aminolevulinic acid in turfgrass leaves (*Lolium perenne* L.) grown in 100% daylight (Light) and 50% daylight (Shade) . Bars show Standard Error of the Mean.

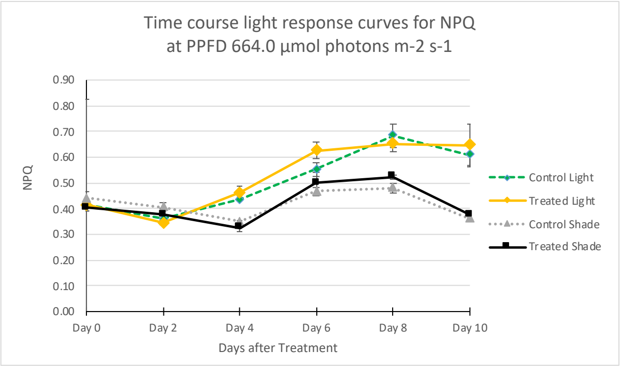
Time course response curves for NPQ (Non-Photochemical Quenching)

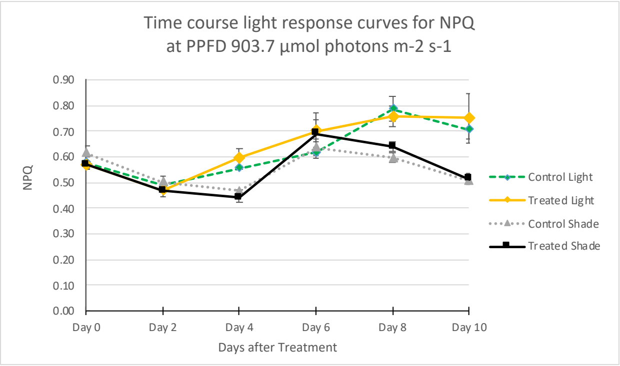
Plants treated and non-treated (Control) with 100mg L^{-1} 5-aminolivulinic acid in 100% daylight (Light) and 50% daylight (Shade). Values show measurements taken at (o (four hours), 2, 4, 6, 8 and 10 days after treatment. PPFD = photosynthetic photon flux density. Bars represent Standard Error of the Mean (n=3)

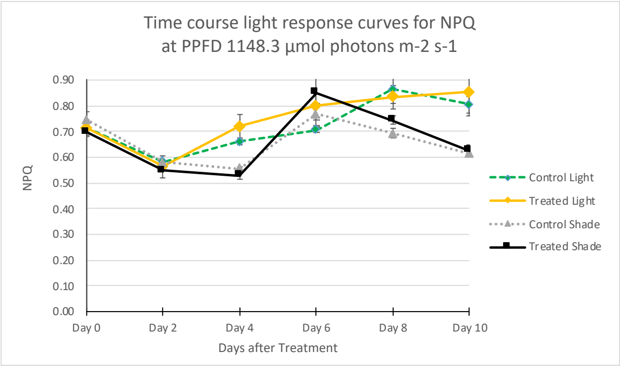












ANOVA for NPQ

DAY 0

Variate: NPQ_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	8.333E-06	8.333E-06	1.00	0.347
Light	1	8.333E-06	8.333E-06	1.00	0.347
ALA.Light	1	8.333E-06	8.333E-06	1.00	0.347
Residual	8	6.667E-05	8.333E-06		
Total	11	9.167E-05			

Variate: NPQ _Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	0.67	0.438
Light	1	0.00003333	0.00003333	0.67	0.438
ALA.Light	1	0.00003333	0.00003333	0.67	0.438
Residual	8	0.00040000	0.00005000		
Total	11	0.00050000			

Variate: NPQ_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000833	0.000833	0.67	0.438
Light	1	0.000133	0.000133	0.11	0.752
ALA.Light	1	0.000300	0.000300	0.24	0.637
Residual	8	0.010000	0.001250		
Total	11	0.011267			

Variate: NPQ_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.001200	0.001200	1.09	0.327
Light	1	0.000133	0.000133	0.12	0.737
ALA.Light	1	0.000833	0.000833	0.76	0.409
Residual	8	0.008800	0.001100		
Total	11	0.010967			

Variate: NPQ_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.001875	0.001875	1.37	0.275
Light	1	0.001008	0.001008	0.74	0.415
ALA.Light	1	0.001008	0.001008	0.74	0.415
Residual	8	0.010933	0.001367		
Total	11	0.014825			

Variate: NPQ_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.001875	0.001875	1.15	0.315
Light	1	0.000208	0.000208	0.13	0.730
ALA.Light	1	0.001875	0.001875	1.15	0.315
Residual	8	0.013067	0.001633		
Total	11	0.017025			

DAY 2

Variate: NPQ_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000000	0.00000000	0.00	1.000
Light	1	0.00003333	0.00003333	1.00	0.347
ALA.Light	1	0.00000000	0.00000000	0.00	1.000
Residual	8	0.00026667	0.00003333		
Total	11	0.00030000			

Variate: NPQ_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000333	0.0000333	0.13	0.729
Light	1	0.0065333	0.0065333	25.29	0.001
ALA.Light	1	0.0005333	0.0005333	2.06	0.189
Residual	8	0.0020667	0.0002583		
Total	11	0.0091667			

Variate: NPQ_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0004083	0.0004083	0.61	0.456
Light	1	0.0520083	0.0520083	78.01	<.001
ALA.Light	1	0.0000750	0.0000750	0.11	0.746
Residual	8	0.0053333	0.0006667		
Total	11	0.0578250			

Variate: NPQ_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0016333	0.0016333	2.72	0.138
Light	1	0.0048000	0.0048000	8.00	0.022
ALA.Light	1	0.0001333	0.0001333	0.22	0.650
Residual	8	0.0048000	0.0006000		
Total	11	0.0113667			

Variate: NPQ_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0024083	0.0024083	2.86	0.129
Light	1	0.0000750	0.0000750	0.09	0.773
ALA.Light	1	0.0002083	0.0002083	0.25	0.632
Residual	8	0.0067333	0.0008417		
Total	11	0.0094250			

Variate: NPQ_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.002133	0.002133	1.50	0.256
Light	1	0.000300	0.000300	0.21	0.659
ALA.Light	1	0.000133	0.000133	0.09	0.767
Residual	8	0.011400	0.001425		
Total	11	0.013967			

DAY 4

Variate: NPQ_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000833	0.00000833	0.50	0.500
Light	1	0.00007500	0.00007500	4.50	0.067
ALA.Light	1	0.00000833	0.00000833	0.50	0.500
Residual	8	0.00013333	0.00001667		
Total	11	0.00022500			

Variate: NPQ_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.06	0.820
Light	1	0.0006750	0.0006750	4.50	0.067
ALA.Light	1	0.0002083	0.0002083	1.39	0.272
Residual	8	0.0012000	0.0001500		
Total	11	0.0020917			

Variate: NPQ_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000133	0.000133	0.07	0.799
Light	1	0.053333	0.053333	27.83	<.001
ALA.Light	1	0.000300	0.000300	0.16	0.703
Residual	8	0.015333	0.001917		
Total	11	0.069100			

Variate: NPQ_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.01	0.914
Light	1	0.0374083	0.0374083	55.42	<.001
ALA.Light	1	0.0018750	0.0018750	2.78	0.134
Residual	8	0.0054000	0.0006750		
Total	11	0.0446917			

Variate: NPQ_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000133	0.000133	0.11	0.750
Light	1	0.045633	0.045633	37.25	<.001
ALA.Light	1	0.003333	0.003333	2.72	0.138
Residual	8	0.009800	0.001225		
Total	11	0.058900			

Variate: NPQ_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000833	0.000833	0.46	0.516
Light	1	0.064533	0.064533	35.69	<.001
ALA.Light	1	0.005633	0.005633	3.12	0.116
Residual	8	0.014467	0.001808		
Total	11	0.085467			

DAY 6

Variate: NPQ_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	8.333E-08	8.333E-08	1.00	0.347
Light	1	8.333E-08	8.333E-08	1.00	0.347
ALA.Light	1	8.333E-08	8.333E-08	1.00	0.347
Residual	8	6.667E-07	8.333E-08		
Total	11	9.167E-07			

Variate: NPQ_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0010083	0.0010083	2.20	0.176
Light	1	0.0002083	0.0002083	0.45	0.519
ALA.Light	1	0.0000083	0.0000083	0.02	0.896
Residual	8	0.0036667	0.0004583		
Total	11	0.0048917			

Variate: NPQ_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.003675	0.003675	2.38	0.161
Light	1	0.161008	0.161008	104.44	<.001
ALA.Light	1	0.001408	0.001408	0.91	0.367
Residual	8	0.012333	0.001542		
Total	11	0.178425			

Variate: NPQ_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.008533	0.008533	2.94	0.125
Light	1	0.034133	0.034133	11.77	0.009
ALA.Light	1	0.001200	0.001200	0.41	0.538
Residual	8	0.023200	0.002900		
Total	11	0.067067			

Variate: NPQ_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.014008	0.014008	1.93	0.202
Light	1	0.000075	0.000075	0.01	0.922
ALA.Light	1	0.000675	0.000675	0.09	0.768
Residual	8	0.058133	0.007267		
Total	11	0.072892			

Variate: NPQ_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.02341	0.02341	2.02	0.193
Light	1	0.00908	0.00908	0.78	0.402
ALA.Light	1	0.00007	0.00007	0.01	0.938
Residual	8	0.09253	0.01157		
Total	11	0.12509			

DAY 8

Variate: NPQ_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00003333	0.00003333	2.00	0.195
Light	1	0.00013333	0.00013333	8.00	0.022
ALA.Light	1	0.00000000	0.00000000	0.00	1.000
Residual	8	0.00013333	0.00001667		
Total	11	0.00030000			

Variate: NPQ_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000083	0.0000083	0.01	0.917
Light	1	0.0070083	0.0070083	9.67	0.014
ALA.Light	1	0.0000750	0.0000750	0.10	0.756
Residual	8	0.0058000	0.0007250		
Total	11	0.0128917			

Variate: NPQ_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000033	0.000033	0.02	0.903
Light	1	0.083333	0.083333	39.53	<.001
ALA.Light	1	0.005633	0.005633	2.67	0.141
Residual	8	0.016867	0.002108		
Total	11	0.105867			

Variate: NPQ_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000133	0.000133	0.05	0.824
Light	1	0.083333	0.083333	33.00	<.001
ALA.Light	1	0.004033	0.004033	1.60	0.242
Residual	8	0.020200	0.002525		
Total	11	0.107700			

Variate: NPQ_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000133	0.000133	0.04	0.846
Light	1	0.070533	0.070533	21.37	0.002
ALA.Light	1	0.004033	0.004033	1.22	0.301
Residual	8	0.026400	0.003300		
Total	11	0.101100			

Variate: NPQ_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000300	0.000300	0.07	0.791
Light	1	0.050700	0.050700	12.65	0.007
ALA.Light	1	0.004800	0.004800	1.20	0.306
Residual	8	0.032067	0.004008		
Total	11	0.087867			

DAY 10

Variate: NPQ_Lss1
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.00000000	0.00000000	0.00	1.000
Light	1	0.00003333	0.00003333	2.00	0.195
ALA.Light	1	0.00000000	0.00000000	0.00	1.000
Residual	8	0.00013333	0.00001667		
Total	11	0.00016667			

Variate: NPQ_Lss2
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.0000750	0.0000750	0.12	0.743
Light	1	0.0494083	0.0494083	76.01	<.001
ALA.Light	1	0.0000083	0.0000083	0.01	0.913
Residual	8	0.0052000	0.0006500		
Total	11	0.0546917			

Variate: NPQ_Lss3
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.000008	0.000008	0.00	0.962
Light	1	0.500208	0.500208	144.99	<.001
ALA.Light	1	0.000075	0.000075	0.02	0.886
Residual	8	0.027600	0.003450		
Total	11	0.527892			

Variate: NPQ_Lss4
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.001875	0.001875	0.29	0.604
Light	1	0.200208	0.200208	31.04	<.001
ALA.Light	1	0.000408	0.000408	0.06	0.808
Residual	8	0.051600	0.006450		
Total	11	0.254092			

Variate: NPQ_Lss5
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.001875	0.001875	0.23	0.647
Light	1	0.143008	0.143008	17.28	0.003
ALA.Light	1	0.001008	0.001008	0.12	0.736
Residual	8	0.066200	0.008275		
Total	11	0.212092			

Variate: NPQ_Lss6
Yellow highlight indicates significant differences at P≤ 0.05

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
ALA	1	0.003008	0.003008	0.39	0.552
Light	1	0.130208	0.130208	16.68	0.004
ALA.Light	1	0.001008	0.001008	0.13	0.729
Residual	8	0.062467	0.007808		
Total	11	0.196692			