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Ocean Glas Impact on Turf

Project Aim & Objectives:

This project was designed to populate information on *Poa annua*, *Agrostis capillaris*, & *Lollium perenne L.* in a controlled pot experiment in response to the product Ocean Glas. Specifically, the project was designed to

1. **Assess root development**
2. **Assess Clipvol**
3. **Assess chlorophyll levels**
4. **Assess proline levels**
5. **Assess drought response**

Methodology:

A natural inoculum pot trial was established as follows; fresh soil cores (approximately 1.8cm diameter, 15cm deep) were collected from a local well-established *Poa annua*, *Agrostis capillaris*, & *Lollium perenne L.* dominant swards and completely homogenised separately by hand. The homogenised cores of each turf type were thoroughly mixed in a 1:9 ratio with sand thereby diluting the natural microbial input and the mixture was divided across 15cm(diameter) x 30cm(d) circular pot sleeves secured by a mesh on the bottom to permit drainage. The pots were watered to approximately 60% field capacity and sown with *Poa annua Agrostis capillaris*, & *Lollium perenne L.* respectively at a rate of approximately 7, 5, & 2 seeds per cm^2. A light sand topdressing (approximately 1mm) was applied, polythene was placed over the pots for 7 days before removal to encourage even germination. The pots were positioned in natural light between 14°C & 16°C throughout the experiment. Pots were maintained thereafter at ~25% FC. Turf was watered as standard and trimmed once per week recording the weight of the clippings at the end of the treatments.

Treatments started 32 days post-sowing on 23rd September 2022 in the early evening out of direct sunlight. Table 1 describes the product & application rates assessed in this project. A number of phenotypes were scored at the time of treatment (non-destructive only, Day0) & on Day21 (post-treatment start) including root dry weight (air dried for 7 days; n= 4), Clipvol (fresh weight of clippings; n = 4), total chlorophyll (as per Palta, 1990; n = 4), & Proline (as per Carillo & Gibbon, 2011; n = 4), which were measured using the same clippings.

On Day22, 15th October 2022, half of the pots were watered to 90% FC and then water was withheld until significant wilting was observed (Day 33). On Day 33 clippings were taken for Proline quantification & the turf was re-watered. Recovery was assessed via Turf Analyser (version 1.0.4).

Figures were generated & statistical analysis was performed using a suite of packages in RStudio (version 1.3.1073) & GraphPad Prism (version 9.1.0).

	Product	**Product Application rate (L/ha* or g/m²**)**	**Application Volume (L/ha)**	**Application Notes**
1	Ocean Glas	20*	300	No viscosity, particulate, or pipetting issues. *Apply every 7 days, 3 applications.

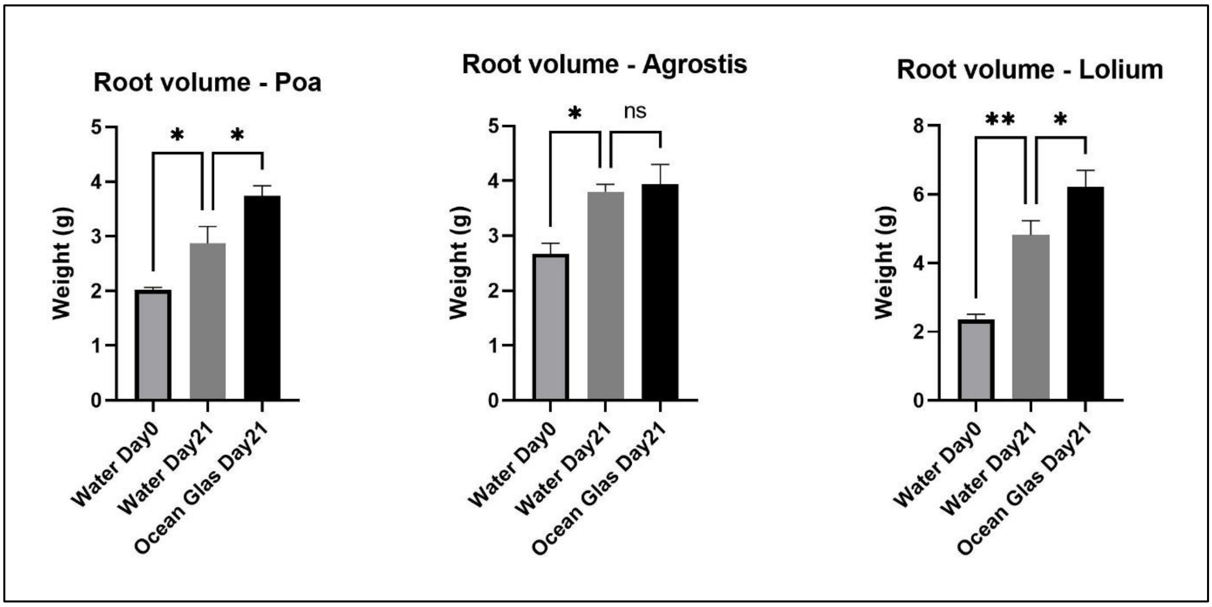
Table 1: PRJ22031 product application overview.

Detailed Summary of Key Findings:

Ocean Glas Treatment resulted in significantly increased root growth in *Poa* & *Lolium* relative to a watered control:

All three turf types treated with Ocean Glas showed a trend of increased root growth generally. While it is noted that the subtle increase in *Agrostis* was not significant, and at the time the turf appeared healthy, upon withholding water it was noted that this turf type declined in both treated & untreated groups (no clear pattern or advantage was observed). Fusarium and Pythium were present in poorly performing pots, and these may have had an effect on root development prior to visual symptoms developing upon stress induction.

The increase in root mass was very clear for both *Poa annua* and *Lolium Perenne*. It is a common observation to find increased mass in the root tissues of plants following exposure to *Ascophyllum* formulations (Ali et al., 2021; Deolu-Ajayi et al., 2022) and has been reported in turf specifically (e.g. Battacharyya et al., 2015; Elansary et al., 2017) therefore these data are consistent with current literature. More robust root systems can generally tolerate higher levels of stress as well as some increased resistance to pathogen pressure (Zhang et al., 2003).



<u>Figure 1</u>: Bar charts summarising root volume expressed as dry weight. Measurements were taken at 21 days post-treatment with exception of the Day0 (pre-treatment) control which was measured on the day of product application. One-way ANOVA; * = p< 0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001

Ocean Glas Treatment had no significant impact on foliar growth relative to a watered control:

While there is a recurring trend of increased foliar mass removed at the end of the trial, the difference was not significant for any turf type. It is possible that the trend is linked to increased root growth (See Figure 1) whereby a larger root system is able to support more vigorous growth above ground, however the main growth specific benefits observed in this trial are below ground.

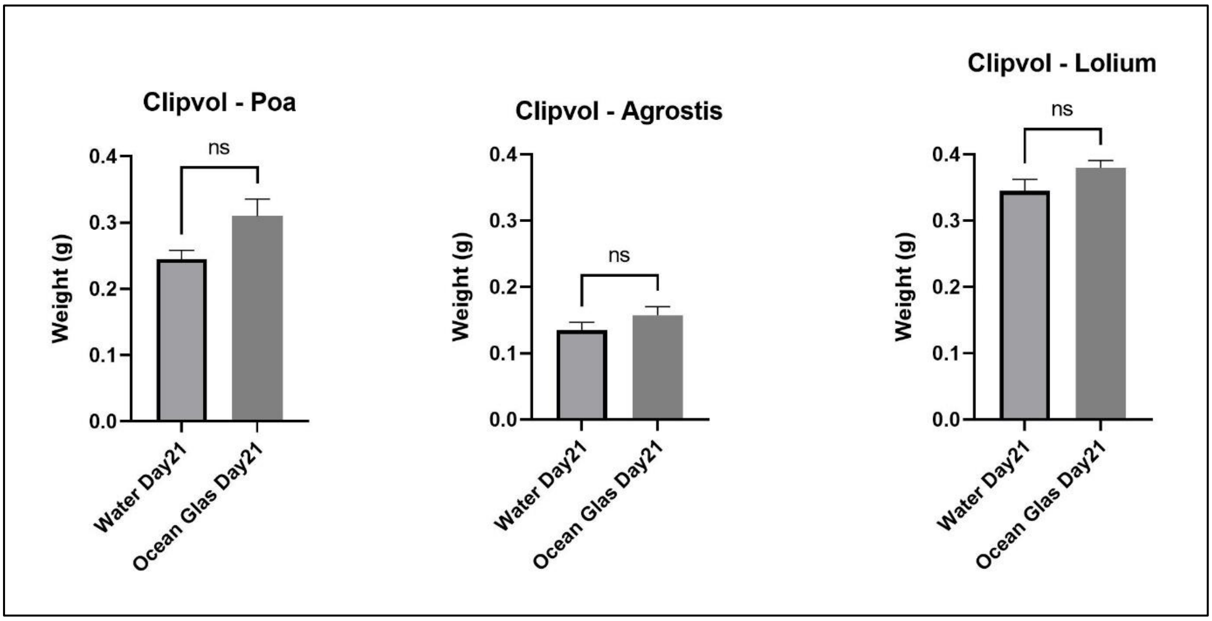


Figure 2: Bar charts summarising foliar growth expressed as fresh weight of clippings on Day21. Un-paired T-test; * = $p< 0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

Ocean Glas Treatment had a significant impact on chlorophyll content in the leaves of *Poa* & *Lolium* relative to a watered control:

The variance in chlorophyll content in Agrostis was high (See Figure 3, middle panel) however a trend of increased chlorophyll concentration, consistent with Poa and Lolium, is evident. The variation in this turf type may be related to the Fusarium and Pythium which only became visible once stress conditions were imposed. The most significant impact was found in the *Poa* which seems to be the most responsive turf type generally to the treatment plan in this trial.

It is common to find subtle, but important, changes in colour following seaweed extract application to turf and some of this may be attributed to increased chlorophyll production, changed rates of photosynthetic pigment turnover, improved photosynthetic efficiency and/or better control of stomatal aperture which permits gas exchange required for photosynthesis (Elansary et al., 2017 & references therein). It is highly likely multiple pathways in these processes are affected by seaweeds. Treatment with seaweed extracts to specifically prime plants against abiotic stress has previously shown similar small increases in chlorophyll concentration under well-watered conditions in grasses (Hosseini et al., 2021, Figure 4a).

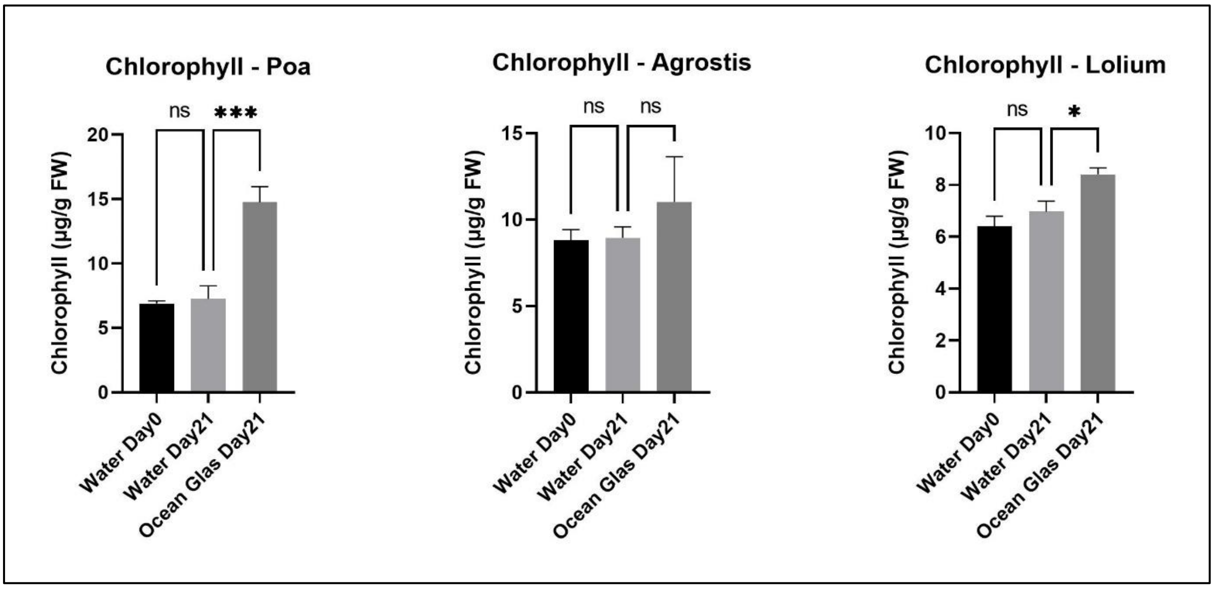


Figure 3: Bar charts summarising chlorophyll content in leaves after three applications on Day21. One-way ANOVA; * = p< 0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

Ocean Glas Treatment had a significant impact on Proline accumulation in the leaves of plants experiencing drought:

Since Proline accumulation in plants is a well-documented 'stress' biomarker (e.g. Jazi et al., 2019), it is not surprising to find that upon introducing drought Proline concentration increased in all turf types. In all turf types Proline concentration was highest in untreated ('un-primed') plants which would strongly suggest those plants were under greater pressure from reduced water availability compared to those which had been 'primed' & treated with Ocean Glas. Plants which had been treated with Ocean Glas showed an increase in Proline concentration compared to levels on Day21 suggesting that they too were responding to the reduced water availability, however the increase was not as high as the untreated plants.

Interestingly, across all three turf types, a small increase in Proline concentration was observed under well-watered conditions in response to three applications Ocean Glas treatment. Other biostimulants including Silicon (Esmaeili et al., 2015, Figure 3) can have a similar effect on Proline levels in some turf types under well-watered conditions although the significance of this remains unclear at this time.

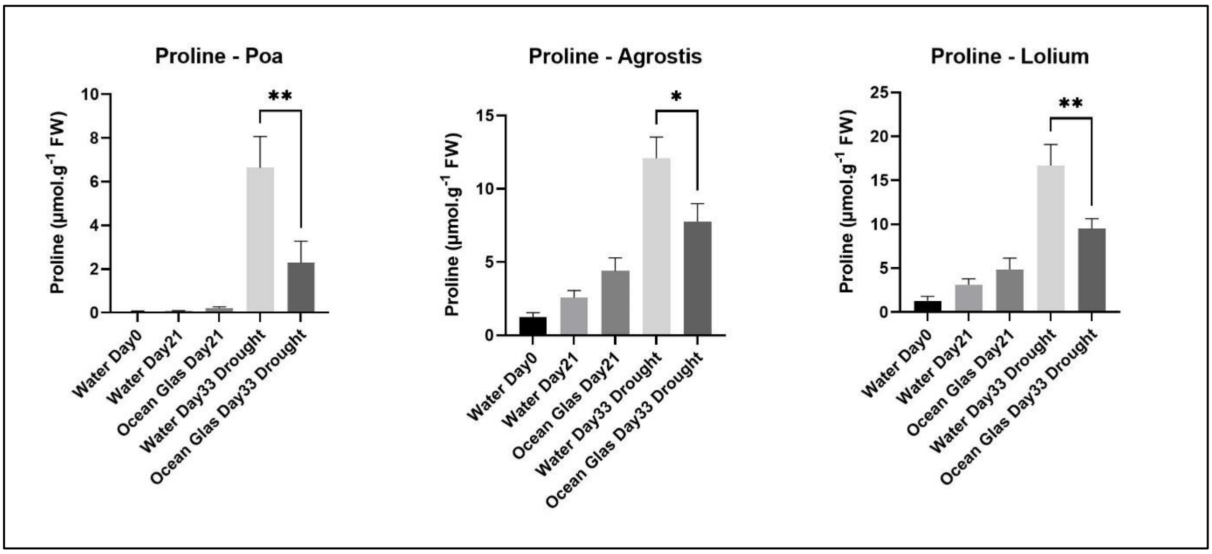


Figure 4: Bar charts summarising Proline content in leaves on Day0, after three applications on Day21, and after drought conditions were created on Day33. One-way ANOVA; * = p< 0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

Ocean Glas Treatment had a significant impact on plants experiencing drought (survival):

All turf types generally responded the same way to water deficit over a ~2 week period; vigour loss & wilting was first noticed in *Agrostis* which had not been treated with Ocean Glas on Day 30, followed by *Poa* & *Lolium* on Day 31. The rate of water loss was slow & gradual due to stormy weather/a lack of sunshine & slightly cooler conditions that expected. Re-watering the pots on Day33 resulted in most plants fully recovering, however recovery was significantly negatively impacted in pots which had not been treated with Ocean Glas in terms of coverage (See Figure 5, top panel). While there was a subtle trend in dark Green Colour Index values in the Poa, these were not significantly different following stress. Many leaf blades appeared darker as wilting progressed however this is mainly an illusion because of water loss concentrating chloroplasts in less physical space & therefore this metric, and chlorophyll measurements, may be best fairly utilised in unstressed turf assessments (see Figure 3).

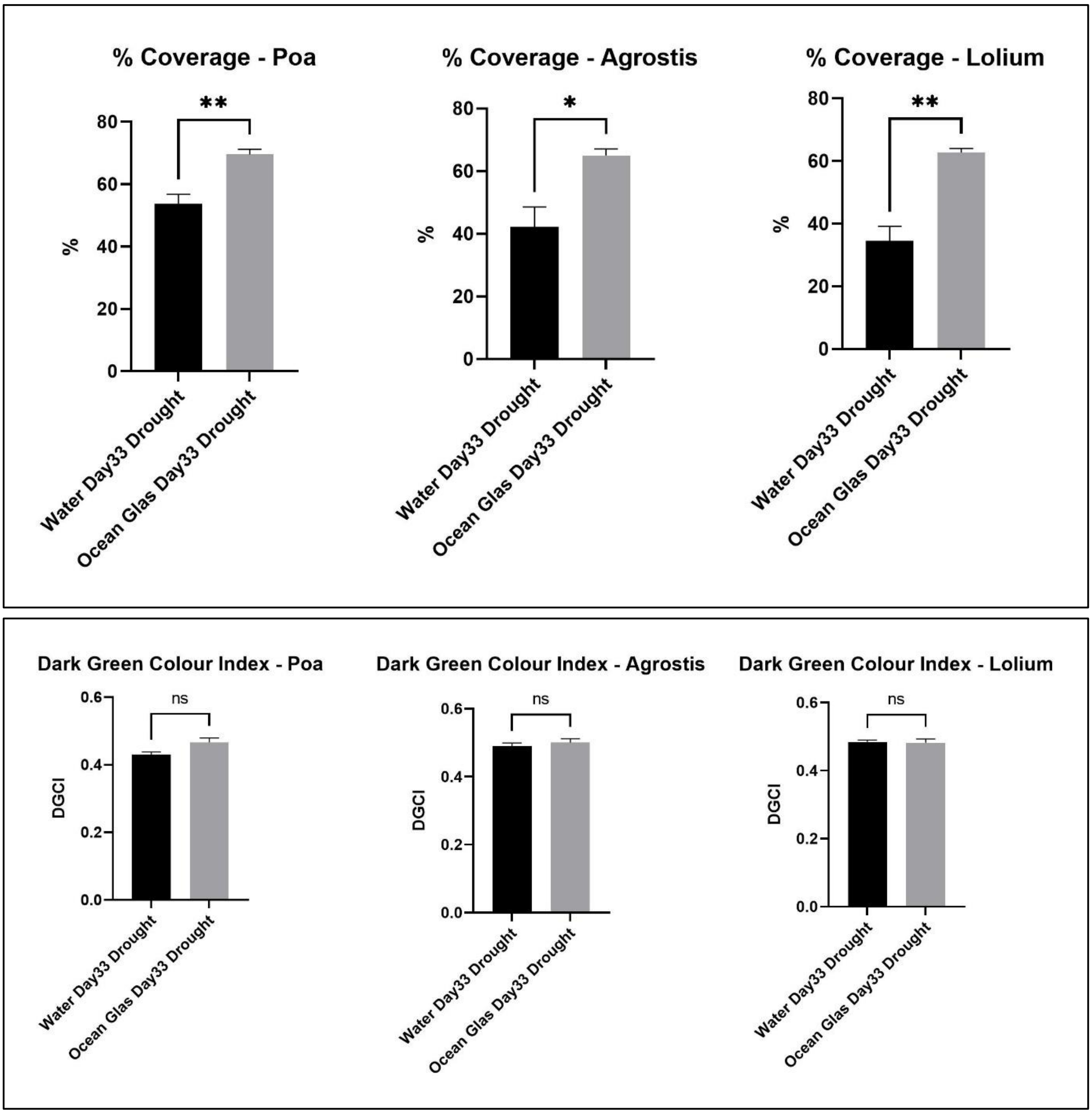


Figure 5: Bar charts summarising % Coverage after drought stress (top panel) & Dark Green Colour Index scores (bottom panel). Un-paired T-test; * = p< 0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

Figure 6: Pots on Day 33 to serve as a visual illustration of coverage & recovery from drought stress. *Agrostis*; top panel. *Lolium*; middle panel. *Poa*; bottom panel.

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