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# Silicon; a benefit or a detriment to plant physiology for Creeping bent (*Agrostis stolonifera* L.), Sheep's fescue (*Festuca ovina* L.) and Perennial ryegrass (*Lolium perenne* L.)?

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**Abstract**

Silicon shows alleviatory effects in many previous studies involving abiotic and biotic stress factors. A germination trial for the optimum concentration of ERA-3 initially showed an optimum dilution factor of 2,500:1 (Distilled water: ERA-3). Over one hundred individuals of each species of *Agrostis stolonifera* L., *Festuca ovina* L. and *Lolium Perenne* L. were all grown for eight weeks in closed hydroponic systems, in warmed greenhouse conditions (15°C). When solely tested, the effects on all three species showed contrasting results to what was expected. In all three selected species, silicon significantly reduced the shoot area, and photosynthetic efficiency (measured as Fv/Fm ratio) as well as reduced tillering. However, predictions of organism density (mass unit$^{-2}$) and pH condition were true, with increased density when silicon was added and acidic and neutral-alkaline for non-silicon and silicon treatments respectively were seen. Further research needs to be conducted on this product, concerning the cellular level characteristics and effects that the ERA-3 silicon has upon grass physiology. The decrease in organism growth and health suggest that the silicon used has toxicity effects.

*Highlights*

- ERA-3 silicon did not promote shoot growth or maintain shoot photosynthetic efficiency.
- ERA-3 silicon caused reduced number of tillers in all three sample species.
- ERA-3 creates a more alkaline environment, needing a non-reacting pH buffer to adjust pH.
- Further research is necessary into cellular level physiology effects of ERA-3 silicon.

*Keywords: Hydroponics, Silicon, Photosynthetic Efficiency, Grass growth.*

## Introduction

*"The facts of silicon (Si) in plant life are one thing; the concepts regarding Si in plant physiology are another thing altogether"* Emanuel Epstein [1].

### Silicon; A brief history

Silicon (Si) is the second most abundant element in the earth's soil (after oxygen) [2,3]. Silicon dioxide ($SiO_2$) more commonly known as silica is an oxidised form of silicon, commonly seen as quartz [4]. Lithology due the type of base rock [5,6] helps determine the quantity of silicon found within the soil [7] due to processes such as silicate weathering [8].

### Silicon in flora

Silicon compounds such as silica accrue in plant tissues, and comprise in many grass forage species between 3-5% of the dry matter [9]. In flora, amorphous silica is absorbed as monosilicic acid ($H_4SiO_4$) [10,11] through the roots [12] and xylem (water-conducting tissues) [13]. The hydrated silica is deposited intra-cellularly as phytoliths (plant opal) within in-fillings of cell walls, intercellular space and lumina [13].

Research of silicon is becoming increasingly seen in the poaceae (or graminae) family. These true grasses comprise of more than approximately ten thousand species within around six hundred genera [14]. Individual taxa within this family that have involved silicon include the Bambusoideae (subfamily) [12,15,16] Pooidae (subfamily) [17] and Oryzeae (tribe) [18]. The genera *Lolium*, *Fescue* and *Agrostis* are still considered to be weakly researched and so this experiment focuses on one species within each of these genera.

Silicon is becoming increasingly important in the alleviative effects of abiotic and biotic stress in flora [2,19,21,22]. Stress factors seen to be alleviated by silicon include heavy metal pollution, salt and drought stress and bacterial and fungicidal disease [2,19,20-22] It is explained by Shi *et al.* [23] that the alleviatory effect from cadmium, is due to the displacement of silicon into the roots and the build up of heavy metals therein. This prevents the deleterious effects of the heavy metal on photosynthetic organelles, and therefore the shoot health and growth.

When concerning crop yield losses due to bacterial infection, silicon has shown to have an increasing socio-economic importance. Bacterial leaf streak caused by *Xanthomonas translucens* in wheat causes yield losses in Brazil of up to 40% [24]. The significant reduction in leaf chlorosis seen by Mehta [24] occurred when Wollastonite (a silica and calcium dominated mineral) was added to local soil samples (see [4]). Mehta [24] therefore suggested the possibility of silicon being used as a natural fertiliser. Therefore any advancement of understanding for this naturally occurring 'fertiliser' would be important to not just science but socio-economic issues throughout the world. This may also be said for alleviation of heavy metal stress, and drought resistance.

The study of silicon effects in hydroponics and the role silicon has in the biochemistry and physiology of higher plants is necessary to be investigated more [20]. Silicon can exist in solution in conditions of essentially a non-polar form below pH7 [10]. Temperature and metabolic inhibitors affect the rate of absorption of silica and nutrients which appear to be independent of transpiration rate in aqueous

solutions [10]. In addition, Barber and Shone [10] showed that the rate of transpiration was slower than that of uptake of silica, which contrasts with nutrient anions and cations. However, it is expected that the silicon in this trial will not have detrimental effects on plant physiology and health, due to the alleviatory effects previously explained (in this section).

### Research aims and hypotheses

Research previously has involved the use of silicon with another experimental factor (such as heavy metal stress). Few studies have investigated silicon uniquely on the effects on plant growth and health. This study aims to investigate the effects of silicon on three species of grass (family Poaceae) within closed hydroponic systems: *Agrostis stolonifera* (L.), *Festuca ovina* (L.) and *Lolium perenne* (L.). It is hypothesized that due to the alleviated effects explained that silicon will promote shoot health (measured as photosynthetic efficiency) and growth (measured using variable techniques – see this issue, section 5.2.2.1: *Grass physiology and morphology*). There will be greater number of leaf tillers in organisms with added silicon (ERA-3) (see section 3.1 Methods: Germination Trial), as well as having a greater root-shoot area ratio (greater shoot growth). Due to the rate of transpiration being slower than the rate of silicon absorption (see [10]) it is also expected the the mass per unit area (density) for organisms grown within the silicon treatment will be greater. It is also expected that the pH of each ERA-3 incorporated solution will tend towards alkalinity, as the nutrient solutions used (see section 5.2.1: *Setup of Equipment and Materials* this issue) is acidic, and the ERA-3 is alkaline at pH12 (Jo Bayley, Personal Communication, 2011).

## Results

### Photosynthetic Efficiency

The anomalies removed from the data sets as explained in section 5.2.3.1 due to not fitting in with the chosen parameters specified are summarised in Table A.1. The results from the Anderson-Darling Normality test on all data sets are shown in Table A.2. With $P<0.005$ for all samples, a two sample t-test occurred within each species. Results showed all species having a significant difference ($P<0.001$) between the photosynthetic efficiency of each organism, showing those with ERA-3 silicon added to have a reduction in photosynthetic efficiency.

### Root-Shoot Area Ratio

With all anomalies removed by Equation B.1, results were shown to be normal with $P<0.005$ for *A. stolonifera* and *L. perenne*, and $P<0.01$ for *F. ovina*. The two-sample t-test for each species had varying but statistically significant results throughout; *A. stolonifera* showed a very strong significant difference of the root-shoot ratio between treatments ($P<0.001$). *L. perenne* showed a strong but weaker significant result than *A. stolonifera* with $P=0.001$. *F. ovina* showed the weakest significant difference in root-shoot area ratios between treatments, with $P<0.05$.

The individuals that were anomalous for the root-shoot ratios mentioned previously had their associated shoot area removed from further testing within each data set. Data were described as nonparametric as *F. ovina* P-values were 0.465 and 0.101 with and without ERA-3 silicon respectively. The Mann-Whitney U test results showed all species having a smaller shoot area when the ERA-3 silicon was added than those with none ($P<0.001$ for all species).

**Table A.1:** Total data count used for Photosynthetic Efficiency (Fv/Fm) measurements, anomalies calculated and removed

| Data set | Total sample size (n) | No. data included: $\left(\bar{x} - \frac{3IQR}{2} < n_{i_{S_j}} < \bar{x} + \frac{3IQR}{2}\right)$ |
|---|---|---|
| $n_{i_{A_1}}$ | 100 | 94 |
| $n_{i_{A_2}}$ | 100 | 95 |
| $n_{i_{F_1}}$ | 100 | 95 |
| $n_{i_{F_2}}$ | 100 | 86 |
| $n_{i_{L_1}}$ | 92 | 90 |
| $n_{i_{L_2}}$ | 71 | 68 |

**Table A.2:** Mean, Standard Deviation and P-Value for each Fv/Fm Photosynthetic Efficiency data set

| Species | Condition | Mean Fv/Fm | Standard Deviation (σ) | P-Value |
|---|---|---|---|---|
| *Agrostis stolonifera* | With ERA-3 Silicon | 0.696 | 0.0721 | X |
| | Without ERA-3 Silicon | 0.800 | 0.0329 | X |
| *Festuca ovina* | With ERA-3 Silicon | 0.720 | 0.0810 | X |
| | Without ERA-3 Silicon | 0.808 | 0.0162 | X |
| *Lolium perenne* | With ERA-3 Silicon | 0.718 | 0.0793 | X |
| | Without ERA-3 Silicon | 0.760 | 0.0599 | X |

## Shoot Area vs. Leaf Tillering

The One Way ANOVA conducted between treatment and number of tillers for each species showed a significant reduction when silicon was added in all species; *A. stolonifera* ($F_{1,198}$=77.10, P<0.001), *F. ovina* ($F_{1,198}$=50.38, P<0.001) and *L. perenne* ($F_{1,161}$=7.70, P=0.006). Figure C.1 shows the descriptive results from the One Way ANOVA. The Two Way ANOVA-GLM results showed all species to have a significant difference in response to shoot area of the number of tillers and the treatment sampled; *A. stolonifera* ($F_{4,194}$=27.78, P<0.001), *F. ovina* ($F_{6,192}$=26.45, P<0.001) and

*L. perenne* ($F_{2,158}$=5.29, P=0.002). Tukey tests for all three species reinforce the significant differences seen for the shoot area and the condition, as P<0.001.

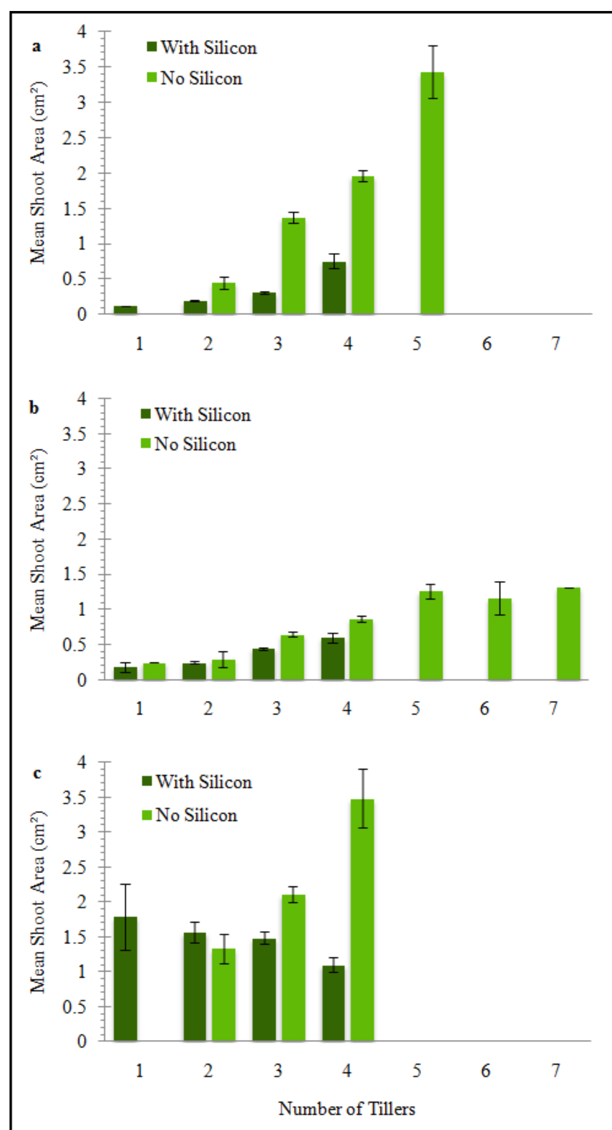


**Figure C.1:** The amount of tillers and the average shoot area of each individual tiller group, for *Agrostis stolonifera* (**a**), *Festuca ovina* (**b**) and *Lolium perenne* (**c**) in treatment with or without ERA-3 silicon

### Wet and Dry Mass

Table A.3 shows all samples with ERA-3 silicon had a smaller average mass per individual than without the silicon treatment.

**Table A.3:** Wet mass and dry mass of total samples from each species and condition

| Species | Condition | No of Individuals | Sum Wet Mass (μg) | Mean Wet Mass Per Individual (μg) | Sum Dry Mass (μg) | Mean Dry Mass Per Individual (μg) | Difference of Mean Wet and Dry Mass (μg) |
|---|---|---|---|---|---|---|---|
| *Agrostis stolonifera* | With Silicon | 100 | 1566 | 15.660 | 623 | 6.230 | 9.430 |
| | No Silicon | 100 | 8523 | 85.230 | 1404 | 14.040 | 71.190 |
| *Festuca ovina* | With Silicon | 100 | 12146 | 121.460 | 1531 | 15.310 | 106.150 |
| | No Silicon | 100 | 12854 | 128.540 | 1489 | 14.890 | 113.650 |
| *Lolium perenne* | With Silicon | 92 | 24274 | 263.848 | 3344 | 36.348 | 227.500 |
| | No Silicon | 71 | 25530 | 359.577 | 2910 | 40.986 | `318.592 |

### pH Analysis

Table A.4 summarizes the pH of each treatment hydroponic tank. The Mann-Whitney U test results show a significant difference between treatments, signifying that the treatment with ERA-3 silicon was more basic in average pH (Median = 7.910) than without silicon which was more acidic (Median = 5.220) with $P=0.0404$ ($P<0.05$).

**Table A.4:** pH analysis of each treatment solution after eight weeks growth.

| Species | Condition | pH |
|---|---|---|
| *Agrostis stolonifera* | Silicon | 7.91 |
| | Control | 5.95 |
| *Festuca ovina* | Silicon | 7.68 |
| | Control | 5.09 |
| *Lolium perenne* | Silicon | 8.3 |
| | Control | 5.22 |

## Discussion

The data recorded generally show a significant negative effect of the ERA-3 silicon on the growth parameters examined. The observations contradict what was expected of the silicon, predicting that silicon significantly increases the size, health and efficiency of the organism, due to the alleviation of biotic and abiotic stresses [2,19,21,22]. These results have only been shown by one other study by Anquan *et al*. [25] involving different levels of silicon dosage testing on maize seedlings in hydroponics.

The photosynthetic efficiency (Fv/Fm) readings were taken on the main stem of each individual, which was decided before results collection. This is an important factor to mention due to the physical appearance of several individuals as exemplified in Figure C.2 of one *F. ovina*. Ellis [26] and Motomura *et al*. [15], both have suggested the hypothesis of silica deposition inhibiting photosynthesis in the leaf mesophyll. Sangster, [27] considered silica to be involved with physiochemical factors, such as transpiration, pH and inorganic and organic matter. Due to transpiration, and the

uptake of silicon as monosilicic acid ($H_4SiO_4$) in flora [11], the flow in the transpiration stream can cause high deposition of silicon at the ends of these pathways in leaf tips and inflorescence bracts [16]. In addition, silicon is said to be non-conspicuous in chlorenchymatous tissue, with reasons why silica prevents photosynthesis not yet known [15].

**Figure C.2:** *Festuca ovina* discolouration of chlorophyll throughout organism, occurring in several individuals

For the root-shoot area ratio, the silicon added treatment showed significantly smaller ratios (similar areas for roots and shoots) due to the shoot area being significantly reduced. This suggests that the silicon causing the reduction in photosynthetic efficiency (leaf health) has also in turn reduced the growth capabilities compared to those without the ERA-3 silicon treatment. This may be due to deposition of hydrated silicon (silica) intra and inter-cellularly [13] which may inhibit photosynthesis in the leaf mesophyll [15,26] as previously explained in this section. However this again is still unknown and further research is necessary on a cellular level to explore if there are differences in composition of cell structure which can help justify why the reduced growth is caused.

It is suggested that the production of fewer larger tillers (as seen in this issue and in Fustec *et al*. [28]) is due to the requirement for more available carbon within the individual plant [29] than the requirement for multiple small tillers. Silicon may have caused a decrease in the number of tillers because of this. Could the silicon (or monosilicic acid) have reacted with the available carbon within each organism, restricting growth? It is suggested that silicification of phytoliths causes carbon to become trapped within these silica bodies [30]. This also helps to explain the reduction in shoot growth if this is indeed the case. Further research is again

necessary concerning the composition of phytoliths within the Poaceae family, and the role they play in physiological and systemic purposes in order to clarify this.

The wet mass-dry mass results showed that the density (mass per unit area) was significantly greater on average per individual with the silicon treatment than without. The atomic weight of silicon is much denser than that of carbon (28.086 and 12.011 respectively). If silicon is therefore actively accumulated through silicon influx and efflux transporter genes Lsi1 and Lsi2 respectively in root cells [18,19] it is possible that silicon has caused this increase in density. Also, if carbon is trapped within the silica bodies [30] causing reduced growth the mass per unit area is also expected to increase. However, the cellular level structure and composition was not investigated in this study and it is suggested that further research into this is necessary for increased understanding.

pH was seen to be more alkaline in treatments with the ERA-3 silicon (mean = pH7.96, $\sigma = 0.313$). Alternatively, the pH of those without the silicon treatment were more acidic (mean = pH5.420, $\sigma = 0.464$). These results may explain the differences in shoot growth as Clark and Zeto [31] studied the effects of micorrhyzal fungi on maize growth in acid and alkaline conditions. It is stated [31] that in soils, physiochemical properties are, or are related to soil pH. Acid (low pH) soils maintain sufficient moisture and are low in soluble salts, having highly weathered clays [32]. Whereas alkaline (high pH) soils are arid and calcareous, having high soluble salt concentrations, poorly weathered clays and sustain sparse or specialised kinds of vegetation [31]. In hydroponics, moisture availability is one hundred per cent; this still might cause an increase in salt stress. However this is contradicted due to the ability of silicon to alleviate salt stress seen in Lee *et al*. (2010) whose work focused on hydroponic systems.

## Conclusions

In hydroponic systems silicon (ERA-3) has shown to significantly reduce shoot health and growth in *A. stolonifera*, *F. ovina* and *L. perenne*, contradicting what was expected. The number of tillers produced also significantly decreased for all species as well as the pH for both treatments being slightly alkaline / acid in treatments with / without silicon (ERA-3).

Further research is necessary in order to understand the cellular level characteristics that may have been a cause of the reduction. In addition, the ERA-3 silicon should be tested in soil conditions to see if the same adverse effects or positive growth and health effects occur.

## Materials and Methods

### Germination Trial

Six petri dishes for each species of grass (*Agrostis stolonifera, Festuca ovina* and *Lolium perenne*) (Emorsgate Seeds, Norfolk) were set up with thirty seeds individually selected by hand in each dish (see Figure C.3). The ERA-3 (Valcent™ Products, Launceston) compound to be tested is 99% silicon metal (containing no added sodium carbonate), is produced by an exothermic process in a digester (Jo Bayley, Personal communication, 2011). This metallurgical grade silicon is over 98% pure, with a potential of hydrogen (pH) of 12.5 (Jo Bayley, Personal communication, 2011). Solutions of distilled water and ERA-3 silicon compound were set up at

different concentrations: 1,000:1, 2,500:1, 5,000:1, 7,500:1 10,000:1 (distilled water: ERA-3 silicon) including a control petri dish containing pure distilled water only for each species. The solutions were then mixed using a flea and magnetic stirrer for thirty seconds at a moderate speed, washing the flea under flowing distilled water after every mix. These standards were retained throughout the germination trial. Two ml of each standard was added to each petri dish three times weekly, whilst during every application of solution a count of the number of germinated seeds took place. Each petri dish was placed in an incubator (Gallenkamp, model IH-150) set at 20°C for the duration of the germination trial set in the dark. The germination trial was considered complete when the total number of seeds germinating remained constant for three days.

**Figure C.3:** Seed Germination Trial set up with conditions: 1,000:1 (1), 2,500:1 (2), 5,000:1 (3), 7,500:1 (4), 10,000:1 (5) and distilled water control (6)

The aim of the germination trial was to find out an optimum concentration of ERA-3 silicon in order to use within the hydroponic system tanks. If a bimodal optimum were to occur, for example, if both 2,500:1 and 7,500:1 dilutions had equal germination rates, the dilution between these two conditions would have been selected.

## Hydroponic System Experiment

*Setup of Equipment and Materials*

The study took place in a temperature-controlled glasshouse in Plymouth (50.37°N, -4.14°E, United Kingdom). Air temperature was maintained around 15°C, with a photoperiod of between 8h35m (max) to 7h49m, over November-January 2010-2011. 6 Active Nutrient Film Technique (NFT) hydroponic tanks (GroTank 205, Nutriculture, Lancashire) were set up with 18 litres of distilled water in each. One model tank for each seed species was used as a control without the silicon supplement, and another 'condition' tank that incorporated the appropriate concentration taken from the preliminary experiment (germination trial). Capillary matting was cut and laid

across each bed and flow pump was attached to circulate the water solutions across the capillary matting. Figure C.4 shows a representation of the set up of hydroponics tanks, with A, B and C indicating the different tanks selected for *Agrostis stolonifera*, *Festuca ovina* and *Lolium perenne* respectively. Both tanks for each species were placed next to each other to reduce environmental effects. The optimum dilution factor attained from the germination trial was 2,500:1. This concentration of ERA-3 silicon was used in each silicon added treatment sample tank.

**Figure C.4:** Hydroponic Tanks set up of *Agrostis stolonifera* (A), *Festuca ovina* (B), and *Lolium perenne* (C)

To each hydroponic tank a trio of commercial nutrient solutions: Flora Micro©, Flora Bloom© and Flora Gro© (GroWell, Warwick) were added weekly: 2ml ea in week one, 3ml ea in week two and 4ml ea per week up to and including weeks three to eight. The change in supply rate was personally advised by a GroWell Technician on the 16th of November 2010. In addition, to the sample tanks, 7.2ml of ERA-3 silicon was added in each. No pH buffer was added as this would increase error for the experiment, and because the ERA-3 silicon is the product being tested, not the ability to find optimal growth conditions for the three grass species.

After eight weeks of growth, the capillary matting in each tank with the organisms rooted within the matting was removed and transferred to a laboratory for physiological analysis. Additionally, a 250ml sample of the solutions left in the hydroponics tanks was removed for pH analysis.

*Analysis*

<u>Grass physiology and morphology</u>
Up to one hundred individuals per tank were randomly selected dependent on germination success within the hydroponics system. Due to concerns with viability of *Lolium perenne* individuals as seen in the germination trial, over one hundred seeds

were sown. This caused the total numbers of germinated individuals in each of the hydroponics tanks for *Lolium perenne* to be ninety-one and seventy-two, with and without ERA-3 silicon respectively. Photosynthetic efficiency measurements were taken using a HandyPEA fluorometer (Hansatech, United Kingdom) via the use of clips with a minimum of 30minutes dark adaptation of the main stem growth. The non-destructive analyses used included: photosynthetic efficiency, morphological external structuring including: leaf area, root area, leaf tillering as well as the wet weight. Samples were grouped into four groups of twenty-five individuals, then wrapped in foil and incubated for a minimum of 48hours at 50°C. Dry weights were then measured.

Morphological external structuring involved the use of an area meter with WinDIAS hardware and software (Dynamax, USA). Each sample was flattened and the total area of the leaves measured. The area of the roots was then taken as well as the number of tillers per organism.

Each organism then had its wet mass measured using a balance scales taking measurements up to 1µg. Specimens were grouped in batches of twenty-five, with any differing sample sizes being noted. Foil that was previously labelled and had their associated mass measured, was used to wrap up the batches of grasses (hereon in known as parcels), which then had the mass measured again (in order to find the wet mass of the grasses within). The parcels were then placed in an incubator at 50°C and dried until constant in mass. The samples were then reweighed and the dry mass calculated. All data were statistically analysed in relation to the aforementioned hypotheses in section 1.3: *Research aims and hypotheses* (this issue).

pH Analysis

The pH of each sample tube was taken using a Basic pH Meter (Denver Instrument Company, Göttingen). The probe was initially calibrated in buffer solutions of pH4 and pH7. After calibration, the probe was placed in each solution and the measurement taken. The probe was then rinsed with distilled water and placed back into the pH4 buffer solution when completed.

*Statistical Analysis*

For the germination trial, no statistical analyses were considered necessary. The optimum concentration used for the hydroponics experiment was 2,500:1 (distilled water: ERA-3) as previously deduced. All data retrieved were tested for their normality using the Anderson-Darling normality test. Subsequently, the appropriate statistical analyses were used, using Microsoft Excel and Minitab 15 mathematical software packages.

Photosynthetic Efficiency

Anomalies were removed from each set of data using the following equation:

$$\left(\bar{x} - \frac{3IQR}{2} < n_{i_{S_j}} < \bar{x} + \frac{3IQR}{2}\right)$$

**Equation B.1:** Removal of anomalous data from individual data sets

where IQR is the Inter-Quartile Range of each data set $n$ with $n_i$ number of individuals included; let $S = \{A_j, F_j, L_j | j = 1,2\}$ (for example, the data set of condition

$j = 1$ in Sample '$A$' or the data set of condition $j = 2$ in Sample L), where '$A$' is *A. stolonifera*, '$F$' is *F. ovina* and '$L$' is *L. perenne*, in condition $j = 1$ with ERA-3 silicon supplement and $j = 2$ without (see Holz *et al*. [33]; this also offers a good understanding of Cardinal Arithmetic and Set Theory).

Therefore let $n_{i_S} = \{n_i := [1{,}100]\}$, (the set size is from values one to one hundred) for example, *A. stolonifera* without ERA-3 silicon is represented as: $n_{i_{A_2}}$. *L. perenne* only had a lower germination success in both samples as explained previously in section 5.2.2.1: *Grass physiology and morphology*. Therefore the cardinality (set size) was lower; $i = [1{,}92]$ with ERA-3 silicon and $i = [1{,}71]$ without.

The Fv/Fm values were then tested for their normality using the Anderson-Darling normality test. All values correlated to a normal distribution, and therefore underwent a two-sample t test.

Root-Shoot Area Ratio

Using WinDIAS (Delta-T Devices, Cambridge) software, the specific root and shoot measurements were taken via flattening the sample onto a back-lit board, and an electronic image being produced. The ratio between root area and shoot area was calculated using Equation B.2:

$$\frac{Root\ area\ (cm^2)}{Shoot\ area\ (cm^2)} \times 100$$

**Equation B.2:** Calculation of the Shoot Area – Root Area Ratio

The ratios produced had anomalies removed using Equation B.1 using the parameters for the data set as with the photosynthetic efficiency values. Data was then tested for normality, and a two-sample t-test was conducted comparing the ratios of each condition within each species.

In order to determine whether the root-shoot area ratios were smaller due to the shoot areas being smaller or root areas being greater, the shoot area data were tested for normality and a Mann-Whitney U test was performed for each species.

Shoot Area vs. Leaf Tillering

The shoot areas and number of leaf tillers were recorded, for all individuals incorporated within each sample. Anomalies became an issue with these data, as from using Equation B.1, the data became non-significant from their previous anomaly-incorporated data set; such that P-Values for *A. stolonifera* and *L. perenne* went from $P<0.005$ to $P>0.05$. Therefore, the data were standardised using Equation B.3:

$$Z = \frac{x - \mu}{\sigma}$$

**Equation B.3:** Standardisation of Shoot Area to a normal distribution.

where $x$ is the value to be standardised, $\mu$ is the mean of the data set and $\sigma$ is the standard deviation of the data set, producing the z-score [34]. 95.44% of data are found within two standard deviations of the mean ($2\sigma$) and 99.74% within $3\sigma$ [35]. After normality testing; the data was transformed to non-normal distributions for all

species. Therefore no anomalies were removed for shoot area as this reduced the significant difference for all data points; $P>0.05$.

An initial One Way ANOVA comparison was carried out between ERA-3 silicon treatment and number of tillers produced. A Two Way ANOVA-GLM (General Linear Model) analysis was then carried out between the shoot area, treatment and number of tillers for each species. The ANOVA was designed to test if the number of tillers produced was affected by the treatment used as well as the relationship this had with the shoot area. The mean and standard error measurements of the shoot area were then plotted against the number of tillers for each species for visual analysis.

Wet and Dry Masses
No statistical analyses were performed on the wet and dry masses of the grass. These recordings were taken to determine whether the difference in treatment caused an increase or decrease in the water content of all sampled individuals. The total mean area measurements for each treatment were calculated. Succinctly, the wet and dry mass per unit area was then calculated for the sum of all individuals in the same sample, using the following equation:

$$\frac{Average\ unit\ mass\ (\mu g)}{Average\ total\ unit\ area\ (cm^2)}$$

**Equation B.4:** Calculation of the average mass per average total unit area

A descriptive comparison then occurred between the outputs from the aforementioned equation.

pH Analysis
Although pH results were limited, a Mann-Whitney U test was conducted between treatments to understand if there was a significant difference between the acidic/basic properties of the samples.

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