Statistical Analysis of Falkland Islands Habitat Restoration Field Trial

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by:
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Summary

This report describes the statistical analysis performed on data collected during a two year field trial in the Falkland Island to assess methods for re-vegetating eroded soil types (with little to no vegetation) using a native seed mixture, established in 2015.

The most significant finding from the trial was that individual and combination treatments of dung, dags and geotextiles applied with the native seed mixture significantly increased the canopy cover, total plant biomass, maximum plant height and number of seedmix species present across all soil types. Of these three treatments, dung was the most effective treatment, followed by dags and then geotextiles. In some instances, combinations of treatments can be more effective than single treatments but the size of the effect is not simply the addition of the individual effects. Treatments were effective across all soil types (clay, peat and sand). It is worth noting that the sand soil type was under-represented in the study, and the site was partially flooded impacting on the application of treatments, so data and conclusions for sand may be unreliable.

Analysis of the data identified three strong colonising species across all eroded soil types: namely, *Elymus magellanicus, Poa flabellata* and *Poa alopecurus* (sand form). Of the remaining species, three were stronger colonisers on specific soil types, namely, *Festuca magellanica* on clay, *Leptinella scariosa* on sand and *Festuca contracta* on peat.

All treatments increased the number of introduced species with dung having the largest impact. However, as dung and dags were all sourced from the same location and no individual non-native species was found across all treatments and sites our result indicate that treatments support colonisation by non-natives found in the

vicinity of eroded areas rather than non-native seeds germinating from applied treatments.

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Experimental Design

A field trial was designed to assess three factors (dung, dags and geotextile (geo)) on three different soil types (peat, clay and sand) across four different regions (Cape Pembroke, Goose Green, Saledero and Fitzroy) on East Falklands. All dung and dags were sourced from the same location.

Each factor had two levels, either the presence or absence of the treatment.

The field trial was of a split plot design with a full factorial of the three factors (8 combinations) used as treatments.

Native seed revegetation trials were established at 16 sites. Each site consisted of 8 plots, 4 of which were randomly allocated to 4 of the 8 treatment combinations. Two were randomly allocated to either a no seed control with all treatments or a no seed control without treatments. The final two plots were allocated to be destructively harvested after either the first or the second full growing season. Each destructively harvested plot was split into 4 quadrants to which the 4 treatment combinations allocated to the plots within the same site were applied. Sites were paired by soil type within a location and together each pair (block) of sites had a complete set of 8 treatment combinations. At Fitzroy, researchers were unable to find a second suitable non-vegetated site for sand and so the site was split into two areas of 8 plots. These two areas were treated as two different sites (13 and 14) in the statistical analysis. All plots except for the no seed controls received the same quantity of native seed mix (10g).

Although the design was implemented as accurately as possible there were occasional deviances that made the design unbalanced and these were taken into account in the statistical analyses. See Appendix 1 for a visual representation of the implemented design.

The sand soil type is under-represented in this study with only two sites (13 and 14) which were unfortunately partially flooded mid-way through the study, impacting the applied treatments so data and conclusions for sand may not be reliable.

In order to monitor rates of surface sediment movement at each site, which could potentially influence seed establishment, sediment traps were installed in February 2015 at each site. These sediment traps were measured on an adhoc basis every 2 to 4 weeks.

Data

Data on environmental conditions (e.g. soil temperature and moisture) and total plant cover were collected throughout the first year of the trial. However, only the data at the end of the study has been analysed and reported here.

Main plots

Primary Measures:
Canopy (%Cover) (for all plant species combined)
Number of Seedmix species present
Number of non-native species present

Secondary Measures:
Bare Ground (%)
Max Height (cms)
Number of Native species present (not in seedmix)
Temperature Average (Average over second year, ^{o}C)
Temperature Range (Range over second year, ^{o}C)
Moisture Average (Average over second year, % volume)
Moisture Range (Range over second year, % volume)
Wind Average (Average over second year, m/s)
Canopy for each seedmix species with maximum cover over 5% (%Cover)

Harvest Plots

Primary Measures:
Biomass (kg/m^2)

Secondary Measures:
Canopy (% Cover)
Max Height (cms)

Statistical Methods

Canopy (%cover), bare ground(%) and canopy (%cover) for each seedmix species are percentages/proportions constrained between 0% and 100% / 0 and 1. These responses were transformed using a logit transformation with an offset of 1/361 prior to analyses (where p is the proportion).

$$\operatorname{logit}(p) = \log\left(\frac{p}{1-p}\right) = \log(p) - \log(1-p).$$

Maximum height and biomass were also transformed by taking logarithms.

Univariate Analysis

All measures were analysed by Analysis of Variance (ANOVA) using Residual Maximum Likelihood (REML)[1] with Genstat[2] software.

Initially a full model was fitted to the data. This is specified as follows:

Fixed effects:

dung, dags and geotextile and all their interactions,
soil and region and their interactions with dungs, dags and geotextile but not their higher order interactions.

Random effects:

Block and site within Block

Soil and region were assessed using the between block variation which was based on two degrees of freedom (df) with a resulting lack of sensitivity. However, the interactions of Region and Soil with other factors, which are more important, are assessed using the within block variation based on much larger dfs.

Secondly, a reduced model was fitted to the data, based on the statistical significance of factors in the full model. This had the same random effects of the full model but only fixed effects with $p<0.05$ in the full model were included. Lower order effects of statistically significant interactions were also kept in the reduced model regardless of their statistical significance. This enabled us to increase the df used for the residual and improve sensitivity of statistical tests.

Residual plots were assessed to check assumptions required for the analysis and for outliers.

Predicted means from the reduced model were extracted along with appropriate standard errors for any statistically significant treatment term. For primary measures the difference between relevant treatments and the significance of the difference along with a 95% CI for the difference were calculated. Additionally, the predicted means for the dung, dags, geotextile three-way interaction were extracted to create interaction plots. If the variable had been transformed then means, differences and confidence intervals were back-transformed to the original scale.

The means of those responses that were transformed using a logit transformation can be back-transformed to p, the proportion, by the following equation:

$$P = \mathrm{logit}^{-1}(\alpha) = \frac{1}{1+\exp(-\alpha)} = \frac{\exp(\alpha)}{\exp(\alpha)+1}$$

Where ɑ is any number on the logit scale.

The back-transformation of the difference between means is the odds ratio.

To investigate natural colonisation and treatment induced colonisation at different sites an additional analysis was performed, again by Analysis of Variance (ANOVA) using Residual Maximum Likelihood (REML), to compare the following groups:

seed but no treatment (from the factorial design),
no seed with no treatment,
no seed with all treatments.

The following model was fitted to the data:
Fixed effects:
treatment
Random effects:
block and site within block

Pairwise comparisons were made with the no seed with no treatment control. The additional control means were added to the interaction plot from the factorial analysis.

The measurements from the sediment traps were collected at adhoc times throughout the year and therefore the quantity collected depends on the period of collection. The cumulative measurement over the period collected for each site was calculated and plotted for different soil types and different regions. The slope of this response curve reflects the rate of sedimentation. Steep curves are times of quick sedimentation, shallow curves are times of slow sedimentation.

Multivariate Analysis

Both Principal Components Analysis (PCA) and Partial Least Squares (PLS)[4] methods were applied to subsets of the response variables using the Simca[3] software.

PCA is a method for reducing the dimensionality of a dataset to visualise and assess underlying trends. It allows us to identify correlations among the measures in the loadings plot and similarities among the plots in the scores plot. PCA does not use the structure of the experimental design, but instead builds a model that explains the most variability between plots. Superimposing information about the design on the scores and loadings plots enables us to interpret the underlying trends.

The loadings plot maps out the weights of the measures in the new components. The first component (x-axis) is the most important one, because it explains the most variability. Two measures that are close to each other are correlated whilst those that are far apart are less so. However, measures that are diagonally opposite to each other on the graph are negatively correlated.

The scores plot maps out the responses of the plots in the new components. Two plots that are close to each other have similar profiles across the original measures whilst those that are far apart have diverse profiles.

PLS is also a method for reducing the dimensionality of a dataset to visualise and assess underlying trends. It has many of the same features as PCA, however, it uses information on the experimental design to build the PLS model and focuses on the dimensions that explain the design features. Superimposing information about the design on the scores and loadings plots aids interpretation.

Two subsets of data were used in the multivariate analysis, the first (Subset 1) focusing on the main measures in the analysis, the second focusing on the flowering of different species.

Subset 1

Measures
Canopy (%Cover)
Bare Ground (%)
Max Height (cms)
Number of Seedmix species present
Number of Introduced species present
Number of Native species present (not in seedmix)
Canopy for each seedmix, native and introduced species (%Cover)
Temperature Average (Average over second year, ^{o}C)
Temperature Range (Range over second year, ^{o}C)
Moisture Average (Average over second year, % volume)
Moisture Range (Range over second year, % volume)
Wind Average (Average over second year, m/s)

Plots
All plots in the factorial design.

Subset 2

Measures
All species that flowered (0/1)
Temperature Average (Average over second year, ^{o}C)
Temperature Range (Range over second year, ^{o}C)
Moisture Average (Average over second year, %)
Moisture Range (Range over second year, %)
Wind Average (Average over second year, m/s)

Plots
All plots in the factorial design

Results

Primary Measures

Canopy (%Cover)

Table 1 Canopy (%Cover), Reduced ANOVA

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Dung	105.31	1	105.31	47	<0.001
Dag	30.75	1	30.75	46.2	<0.001
Dung.Dag	13.19	1	13.19	51	<0.001
Geo	11.39	1	11.39	46.2	0.002
Soil	0.33	2	0.17	4.9	0.851
Dung.Soil	8.85	2	4.43	48	0.017

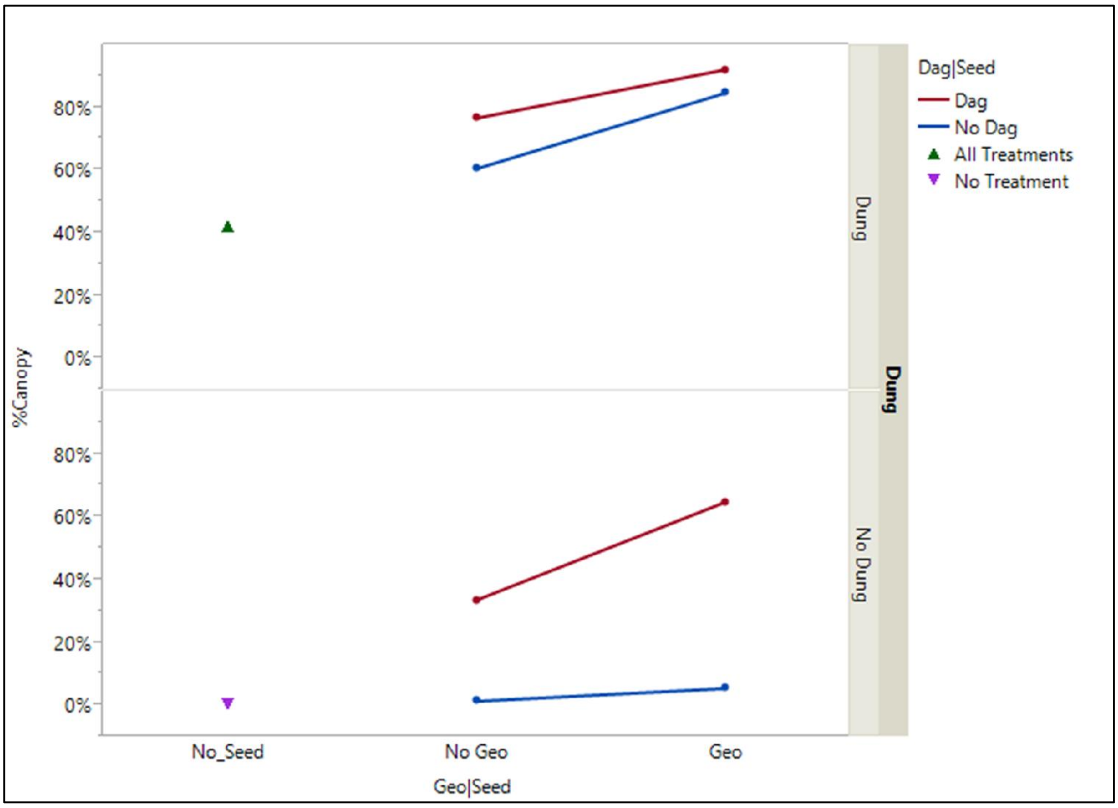
All three main effects for treatments, dung, dags and geotextiles were highly statistically significant (See Table 1).

The predicted means for dung, dags and geotextile combinations are presented in Table 2 and Figure 1. Adding dung alone increases percent cover by 59%, adding dags alone increases percent cover by 32% and adding geotextiles alone increases percent cover by 4%.

Table 2 Canopy (%Cover), Predicted Dung.Dag.Geotextile combination means from reduced model.

		Geo	No Geo
Dung	Dag	92.1%	76.6%
	No Dag	84.6%	60.5%
No Dung	Dag	64.8%	33.6%
	No Dag	5.4%	1.4%

Figure 1 Canopy (%Cover), Predicted Dung.Dag.Geotextile combination means from reduced model with No seed controls.



The interactions between dung and dags, and between dung and soil type were also statistically significant (See Table 1). This indicates that the size of the effect of dung and dags depends on whether the other is present or not.

The effect of the dung and dag combination is statistically significant when compared to the treatment of dags alone (ie. 37% increase, p=0.0016). However, the effect of the dung and dag combination is not statistically significant when compared to the treatment of dung alone (ie. 11.7% increase, p=0.1490) (See Table 3).

Table 3 Canopy (%Cover), Predicted Dung.Dag interaction means from reduced model.

	Dung	No Dung	Difference	p-value
Dag	86.1%	49.1%	37.0%	0.0016
No Dag	74.4%	2.8%	71.6%	<0.0001
Difference	11.7%	46.3%		
p-value	0.1490	<0.0001		

The strength of dung as a treatment, enhancing plant canopy cover, depends on the underlying soil type. Adding dung on peat significantly increases canopy cover over a year by 81.3% (p<0.0001), on clay by 73.7% (p<0.0001), but on sand dung only increased canopy cover by 26.7% (p=0.2797). Although, it is noteworthy that the significance of the dung treatment on sand may be reduced because of the lower level of replication (See Table 4).

Table 4 Canopy (%Cover), Predicted Dung.Soil interaction means from reduced model.

	Clay	Peat	Sand
Dung	81.2%	91.6%	61.6%
No Dung	7.5%	10.3%	34.9%
Difference	73.7%	81.3%	26.7%
p-value	<0.0001	<0.0001	0.2797

The analysis of variance of the controls indicate an overall statistically significant difference between the three control groups (p<0.001). Sowing native seeds without treatments did not significantly increase plant canopy compared to plots without seeds or treatments (p=0.3887). However, applying all the treatments without the native seed mixture significantly increased plant canopy cover by 41.8% compared to plots without seeds or treatments (p<0.001). The predicted means from the control analysis are presented in Table 5 and the no seed controls with and without treatments are included in Figure 1 as a reference.

Table 5 Canopy (%Cover), Predicted control means.

	No Seed, No Treatment	Native seed only	No Seed, All Treatments
Back-transformed Mean	0.3%	0.8%	41.8%
Difference from No Seed, No Treatment.		0.5%	41.6%
p-value		0.3887	<0.0001

Seedmix species present

Table 6 Seedmix species present, Reduced ANOVA

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Dung	73.09	1	73.09	45.8	<0.001
Dag	15.26	1	15.26	45.6	<0.001
Dung.Dag	12.33	1	12.33	49	<0.001
Soil	8.1	2	4.05	4.9	0.092
Dung.Soil	7.02	2	3.51	46.5	0.038

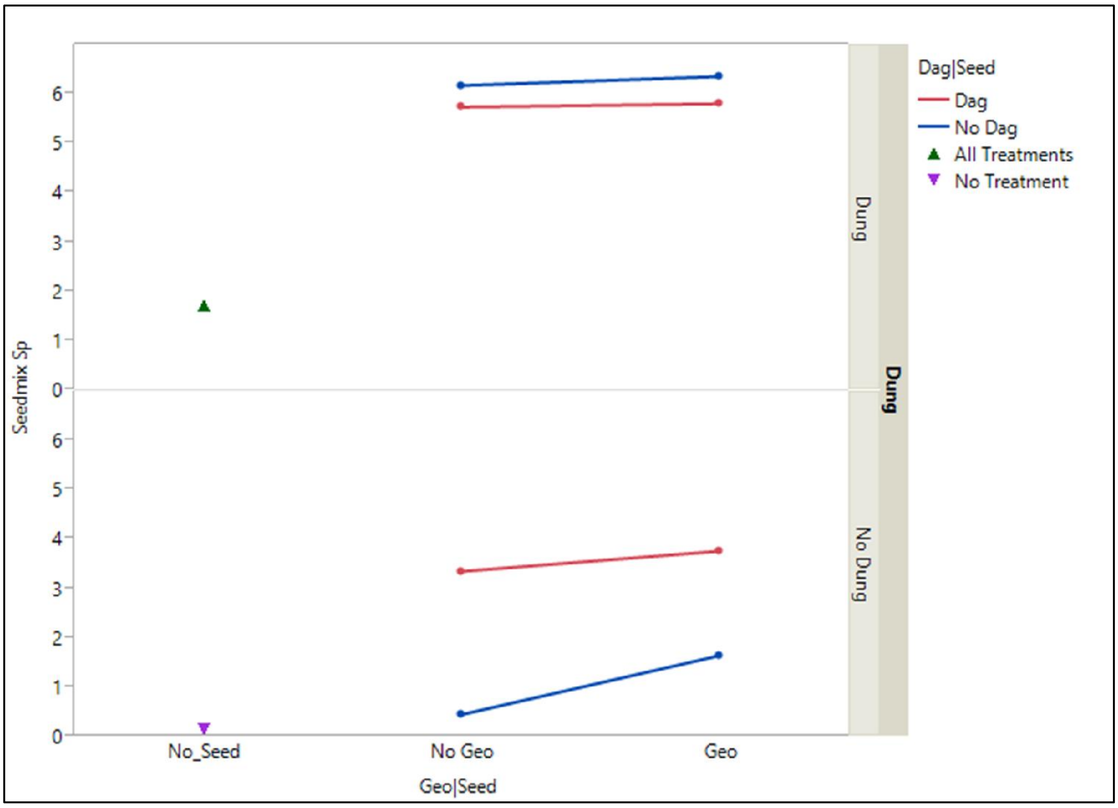
The main effects for dung and dags were highly statistically significant (See Table 6). Geotextile was not statistically significant in the full model (p>0.05) and therefore is not present in the reduced model.

The predicted means for dung, dags and geotextile combinations are presented in Table 7 and Figure 2. Adding dung alone increases the number of seedmix species present by on average 5.7 species and adding dags alone increases the number of seedmix species present by on average 2.8 species.

Table 7 Seedmix species present, Predicted Dung.Dag.Geotextile combination means from reduced model.

		Geo	No Geo
Dung	Dag	5.8	5.7
	No Dag	6.4	6.2
No Dung	Dag	3.8	3.3
	No Dag	1.6	0.5

Figure 2 Seedmix species present, Predicted Dung.Dag.Geotextile combination means from reduced model with No seed controls.



The interactions between dung and dags, and between dung and soil type were also statistically significant (See Table 6). This indicates that the size of the effect of dung and dags depends on whether the other is present or not.

The effect of the dung and dag combination is statistically significant when compared to the treatment of dags alone. The increase is 1.6 species (p=0. 0198). However, the effect of the dung and dag combination is not statistically significant when compared to the treatment of dung alone. Here the increase is 0.3 species (p=0. 5875) (See Table 8).

Table 8 Seedmix species present, Predicted Dung.Dag interaction means from reduced model.

	Dung	No Dung	Difference	p-value
Dag	6.3	4.7	1.6	0.0198

No Dag	6.0	1.3	4.6	<0.0001
Difference	0.3	3.3		
p-value	0.5875	<0.0001		

The strength of dung as a treatment, increasing the number of seedmix species present, depends on the underlying soil type. Adding dung on peat significantly increases the number of seedmix species by 4.2 (p<0.0001), on clay by 4.4 species (p<0.0001), but on sand dung only increased the number of seedmix species by 0.8 (p=0.4995). Although, it is noteworthy that the significance of the dung treatment on sand may be reduced because of the lower level of replication (See Table 9).

Table 9 Seedmix species present, Predicted Dung.Soil interaction means from reduced model.

	Clay	Peat	Sand
Dung	7.2	7.9	3.3
No Dung	2.8	3.8	2.5
Difference	4.4	4.2	0.8
p-value	<0.0001	<0.0001	0.4995

The analysis of variance of the controls indicate an overall statistically significant difference between the three control groups (p<0.001). Sowing native seeds without treatments did not significantly increase the number of seedmix species present compared to plots without seeds or treatments (p=0.0770). However, applying all the treatments without the native seed mixture significantly increased the number of seedmix species present by 1.6 species compared to plots without seeds or treatments (p=0.0021). The predicted means from the control analysis are presented in Table 10 and the no seed controls with and without treatments are included in Figure 2 as a reference.

Table 10 Seedmix species present, Predicted control means.

	No Seed, No Treatment	Native seed only	No Seed, All Treatments
Back-transformed Mean	0.1	1.1	1.7
Difference from No Seed, No Treatment.		1.0	1.6
p-value		0.0770	0.0021

Introduced species present

Table 11 Introduced species present, Reduced ANOVA

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Dung	23.96	1	23.96	47.9	<0.001
Dag	7.43	1	7.43	47.5	0.009
Geo	9.13	1	9.13	47.5	0.004

The main effects for dung, dags and geotextiles were statistically significant (See Table 11).

The predicted means for dung, dags and geotextile combination means are presented in Table 12 and Fig 3.

Adding dung increases the number of introduced species present by on average 1.1 species ($p<0.0001$). By adding dags the number of introduced species is further increased by on average 0.6 species ($p=0.0086$) and this is further increased by on average 0.7 species ($p=0.004$) when geotextile is added. (See Table 12 and 13).

Table 12 Introduced species present, Predicted Dung, Dag and Geotextile combination means from reduced model.

		Geo	No Geo
Dung	Dag	2.5	1.9
	No Dag	1.9	1.2
No Dung	Dag	1.4	0.8
	No Dag	0.8	0.1

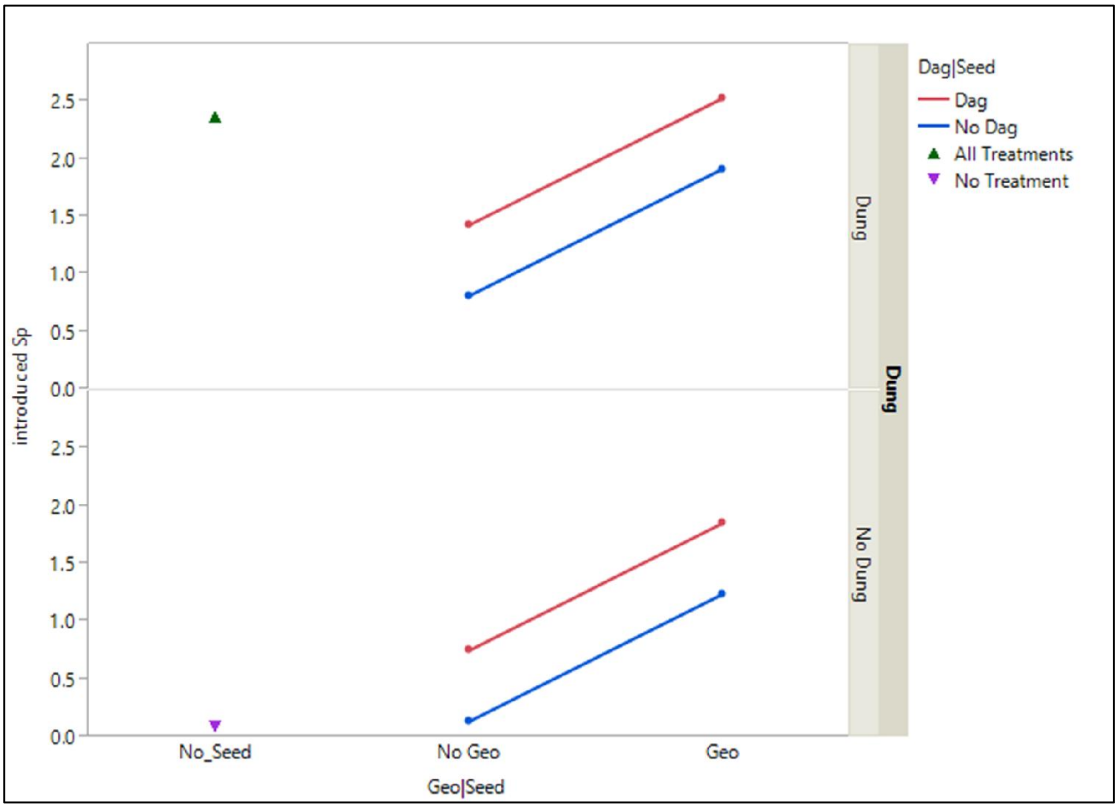
Table 13 Introduced species present, Predicted Dung, Dag and Geotextile means from reduced model.

	Dung
Dung Mean	1.9
No Dung Mean	0.8
Difference	1.1
p-value	<0.0001

	Dag
Dag Mean	1.6
No Dag Mean	1.0
Difference	0.6
p-value	0.0086

	Geotextile
Geo Mean	1.7
No Geo Mean	1.0
Difference	0.7
p-value	0.0040

Figure 3 Introduced species present, Predicted Dung, Dag and Geotextile combination means from reduced model with No seed controls.



No interactions were statistically significant (See Table 11). This indicates that the size of the effect of dung, dags and geotextiles are independent of each other, region and soil.

The analysis of variance of the controls indicate an overall statistically significant difference between the three control groups (p<0.001). Sowing native seeds without treatments did not significantly increase the number of introduced species present compared to plots without seeds or treatments (p=0.5694). However, applying all the treatments without the native seed mixture significantly increased the number of introduced species present by 2.3 species compared to plots without seeds or treatments (p<0.0001). The predicted means from the control analysis are presented in Table 14 and the no seed controls with and without treatments are included in Figure 3 as a reference. Note that the no seed all treatment mean (2.4 species) is very similar to the native seed, all treatments mean (2.5 species).

Table 14 Introduced species present, Predicted control means.

	No Seed, No Treatment	Native seed only	No Seed, All Treatments
Back-transformed Mean	0.1	0.3	2.4
Difference from No Seed, No Treatment.		0.2	2.3
p-value		0.5694	<0.0001

Biomass (Kg/m2)

Table 15 Biomass (Kg/m2), Reduced ANOVA

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Dung	71.54	1	71.54	41.8	<0.001
Dag	40.77	1	40.77	41.9	<0.001
Geo	14.71	1	14.71	41.8	<0.001
Dung.Dag	20.61	1	20.61	45.9	<0.001
Soil	4.61	2	2.3	5	0.195
Dung.Soil	26.93	2	13.47	41	<0.001
Geo.Soil	9.97	2	4.98	41	0.012

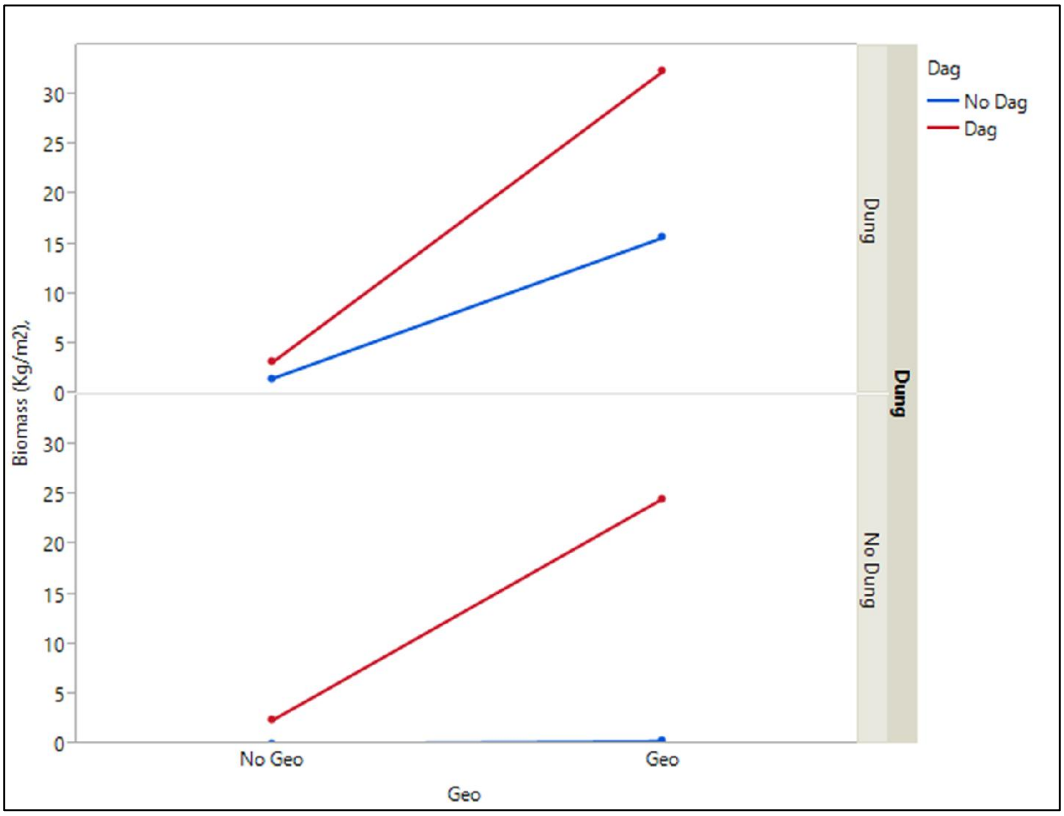
The main effects for dung, dags and geotextiles were highly statistically significant (See Table 15).

The predicted means for dung, dags and geotextile combination means are presented in Table 16 and Figure 4.

Table 16 Biomass (Kg/m2), Predicted Dung.Dag.Geotextile combination means from reduced model.

		Geo	No Geo
Dung	Dag	32.36	3.15
	No Dag	15.67	1.52
No Dung	Dag	24.61	2.39
	No Dag	0.29	0.02

Figure 4 Biomass (Kg/m2), Predicted Dung, Dag and Geotextile combination means from reduced model.



However, the interactions between dung and dags, between dung and soil type and between geotextile and soil type were also statistically significant (See Table 15). This indicates that the size of the effect of dung or dags depends on whether the other is present or not.

The effect of the dung and dag combination is not statistically significant when compared to the treatment of dags alone (p=0.6583) or when compared to dung alone (p=0.2088). (See Table 17).

Table 17 Biomass (Kg/m2), Predicted Dung.Dag interaction means from reduced model.

Dung	Dung	No Dung	Ratio	p-value
Dag	10.10	7.68	1.31	0.6583
No Dag	4.89	0.08	51.95	<0.0001
Ratio	2.06	81.58		
p-value	0.2088	<0.0001		

The effect of dung depends on the soil type. Adding dung significantly increases total plant biomass on peat (p<0.0001) and clay (p<0.0001), whilst on sand application of dung has no significant effect on total plant biomass (p=0.0591) (See Table 18). Note that the biomass data is gathered from harvest plots, one from each site. Therefore, the 8 sand biomass data points are gathered from just two plots and thus are highly vulnerable to untoward effects. Partial flooding of the sand sites is likely to have reduced the growing duration and conditions for harvest plots resulting in different results between treatment effects on plant cover and biomass production at the sand site

Table 18 Biomass (Kg/m2), Predicted Dung.Soil interaction means from reduced model.

	Clay	Peat	Sand
Dung	26.52	73.34	0.17
No Dung	0.34	1.30	1.32
Ratio	74.77	56.16	0.13
p-value	<0.0001	<0.0001	0.0591

Similarly, the effect of geotextile depends on the soil type. Adding geotextile on sand significantly increases total plant biomass (p=0.0001) whilst it makes no significant impact on clay (p=0.0968) or on peat (p=0.0748) (See Table 19). This may seem strange as the mean for the geotextile on clay and peat are a similar size, if not larger than that on sand. However, the baseline of 0.03 of no geotextile is lower on the sand than the other soil types, exaggerating the effect of the geotextile.

Table 19 Biomass (Kg/m2), Predicted Geo.Soil interaction means from reduced model.

Soil	Clay	Peat	Sand
Geo	4.96	17.70	5.46
No Geo	1.88	5.40	0.03
Ratio	2.62	3.27	125.31
p-value	0.0968	0.0748	0.0001

There were no control measures of biomass.

Secondary Measures

Summaries of the secondary measures' results from the ANOVA alongside the primary measure results are presented in tables:

20. Main plot measures
21. Canopy for individual seedmix species
22. Harvest plot measures

Note that the number of statistical tests across all the variables is substantial (15 test * 25 variables=375). As we are testing at the 5% level of significance we can expect 1 in 20 results to be false positive results ie. 19 out of 375. Therefore, some of the inconsistent effects may be spurious in nature.

Very similar patterns were seen in the analysis of both bare ground and height as in the analysis of canopy.

Environmental measures were much more related to region and soil than other measures as they act on a macro level. However, some treatment effects were observed, in particular geotextile, and to a lesser extent dags, which appear to have created micro-climates.

The number of non-seedmix native species colonising the plots was not affected by any of the treatments.

The analyses of canopy for individual seedmix species reflected the patterns seen in the overall canopy measure. However, the size of effects is generally reduced as the impact of treatment is diluted by the splitting into separate species.

The canopy and height measures from the harvest plots show a very similar pattern of effects as the biomass measure.

All interaction plots of secondary measure are presented in Figures 5a, 5b and 5c.

Table 20 Summary of main plot measures' ANOVAs

Fixed term	Logit Canopy	Logit Bare ground	Log Height	Ave wind	Moist Ave	Moist Range	Temp Ave	Temp Range	Seedmix sp.	Native sp.	Introduced sp.
Dung	<0.001	<0.001	<0.001			0.017	0.716	0.207	<0.001		<0.001
Dag	<0.001	<0.001	<0.001	0.312			<0.001	<0.001	<0.001		0.009
Geo	0.002	0.003	0.383	0.202	<0.001	0.008	<0.001	<0.001			0.004
Dung.Dag	<0.001	<0.001	<0.001				0.019	0.049	<0.001		
Dung.Geo			0.796								
Dag.Geo			0.686								
Dung.Dag.Geo			0.066								
Soil	0.851	0.846		0.927	0.008	0.154	0.014	0.018	0.092		
Region				0.183	0.022	0.678					
Dung.Soil	0.017	0.002							0.038		
Dag.Soil				0.015							
Geo.Soil		0.021			<0.001	<0.001					
Dung.Region											
Dag.Region											
Geo.Region				0.12	0.006	0.017					

Key
p<0.1 (red)
0.05<p<0.01 (orange)
P<0.05 in full model (yellow)
Primary Measures (blue)

Table 21 Summary of individual seedmix species canopy measures' ANOVAs

Fixed term	logit Poa flabellata	logit Elymus magellanicus	logit Poa alopecurus (Sand type)	logit Hierochloe redolens	logit Trisetum phleoides	logit Festuca magellanica	logit Poa alopecurus (Peat type)	logit Leptinella scariosa	logit Deschampsia flexuosa	logit Festuca contracta	logit Juncus scheuchzerioides
Dung	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.13	0.088
Dag	0.014	<0.001	0.005	0.041		0.012	0.032	0.789			0.992
Geo	0.788	0.576	0.722	0.019			0.703	0.303		0.674	
Dung.Dag	0.001	<0.001		0.051				0.047			0.053
Dung.Geo				0.015			0.002			0.041	
Dag.Geo		0.018						0.033			
Dung.Dag.Geo											
Soil	0.081		0.613	0.074			0.202				
Region	0.048							0.505			0.084
Dung.Soil	0.091										
Dag.Soil				0.017			0.04				
Geo.Soil	0.062		0.045	0.006							
Dung.Region	0.037										0.027
Dag.Region								0.041			
Geo.Region											

Key
p<0.1
0.05<p<0.01
P<0.05 in full model

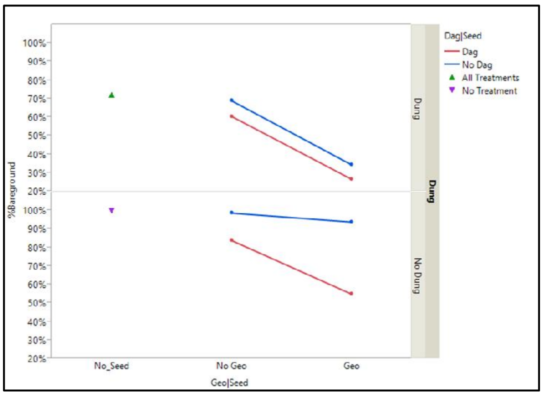
Table 22 Summary of harvest plot measures' ANOVAs

Fixed term	LogitCanopy	LogHeight	LogBiomass
Dung	<0.001	<0.001	<0.001
Dag	<0.001	<0.001	<0.001
Geo	<0.001	0.004	<0.001
Dung.Dag	0.01		<0.001
Dung.Geo			
Dag.Geo			
Dung.Dag.Geo			
Soil	0.411	0.107	0.195
Region			
Dung.Soil	<0.001	0.006	<0.001
Dag.Soil			
Geo.Soil	0.008	0.028	0.012
Dung.Region			
Dag.Region			
Geo.Region			

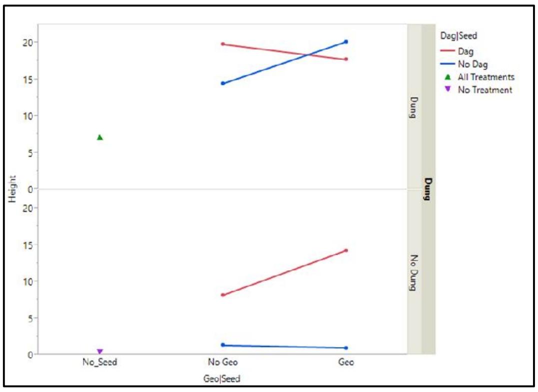
Key
p<0.1
0.05<p<0.01
P<0.05 in full model

Fig.5a Interaction plots for Secondary measures

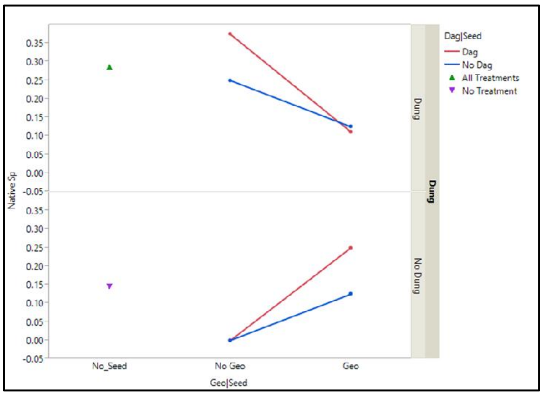
Bareground



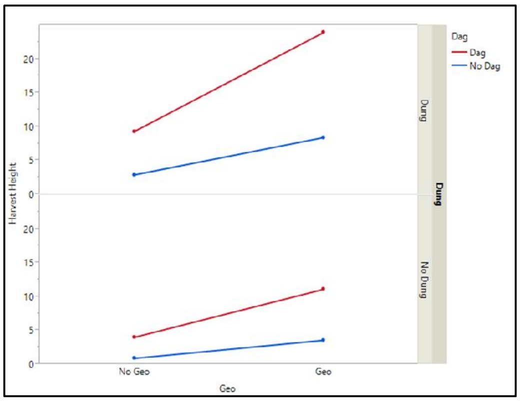
Max. Height



Native Sp



Harvest Height



Harvest Canopy

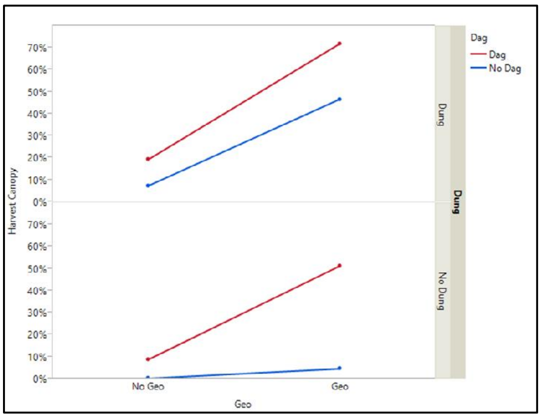
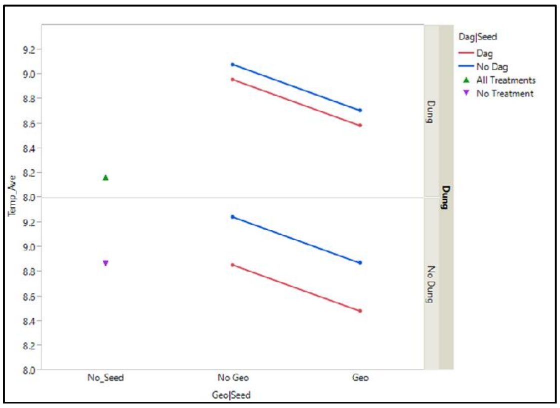
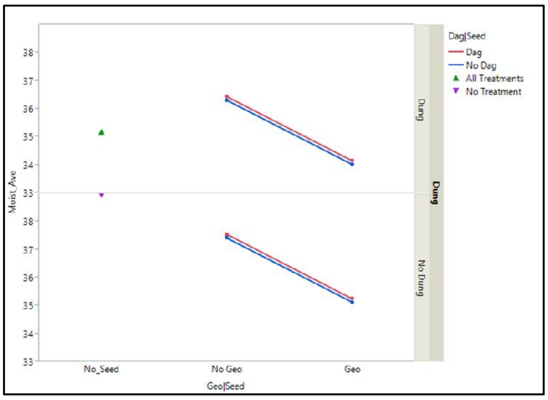


Fig.5b Interaction plots for Environmental measures

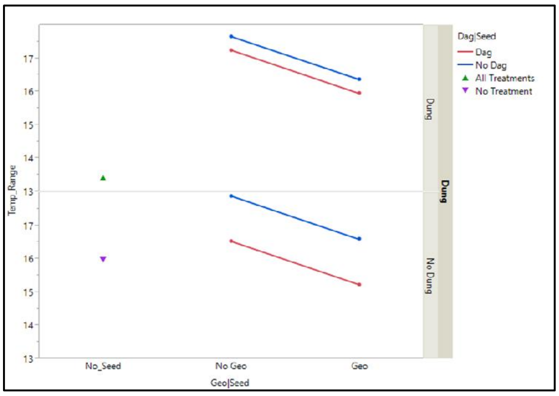
Temperature Average



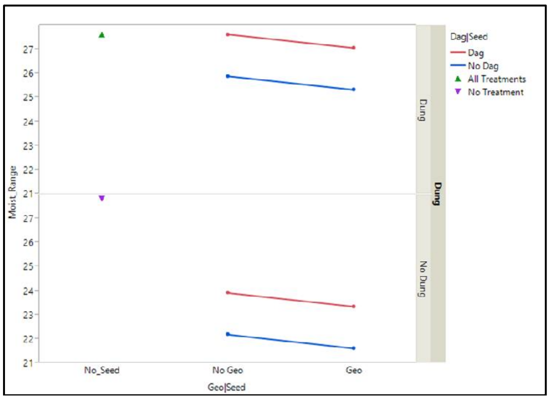
Moisture Average



Temperature Range



Moisture Range



Average wind

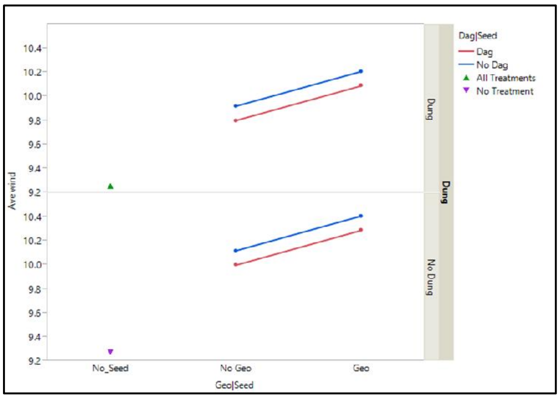
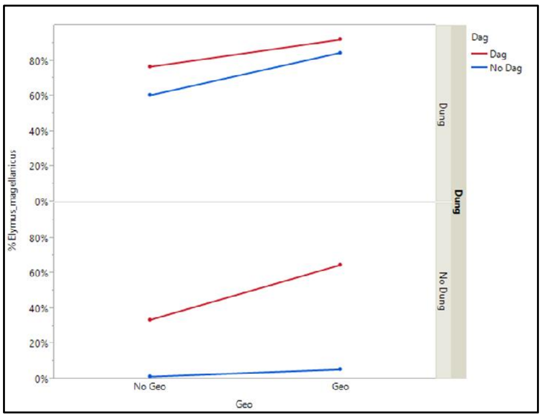
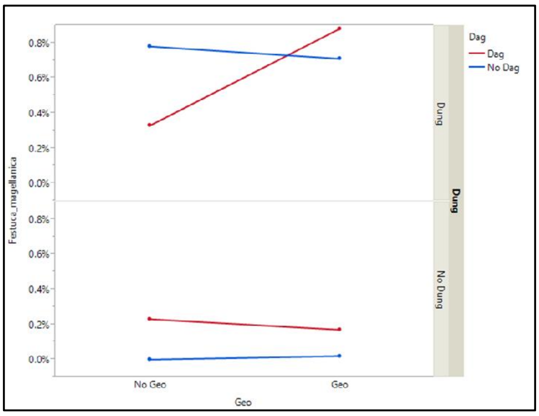


Fig.5c Interaction plots for Canopy of individual Seedmix species.

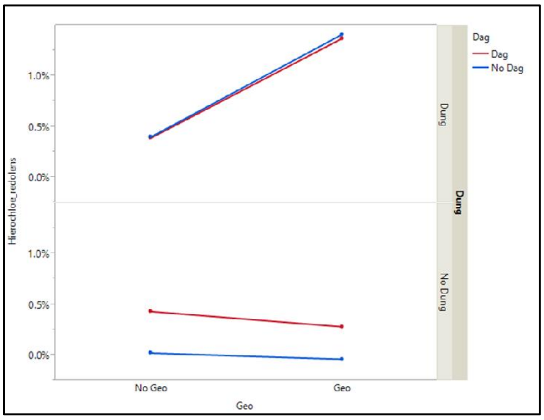
Elymus magellanicus



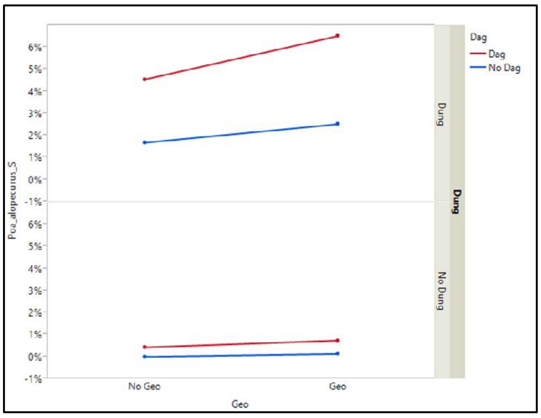
Festuca magellanica



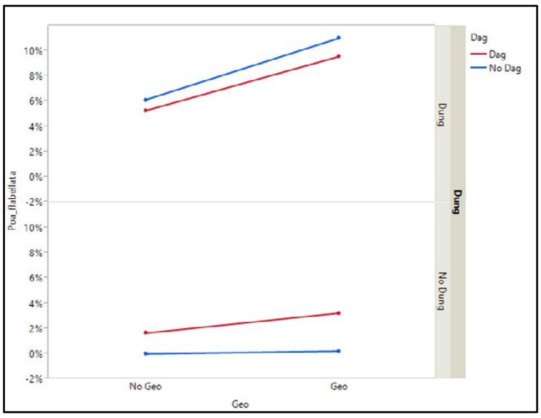
Hierochloe redolens



Poa alopecurus S



Poa flabellata



Trisetum phleoides

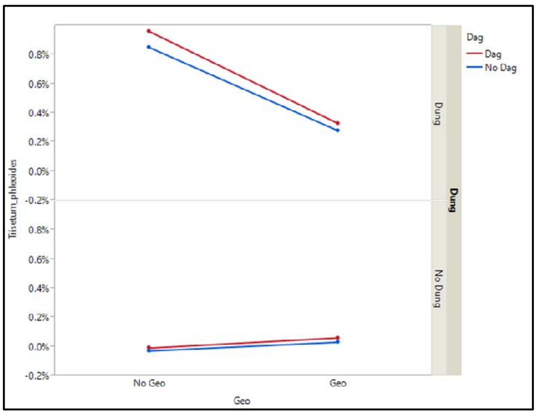
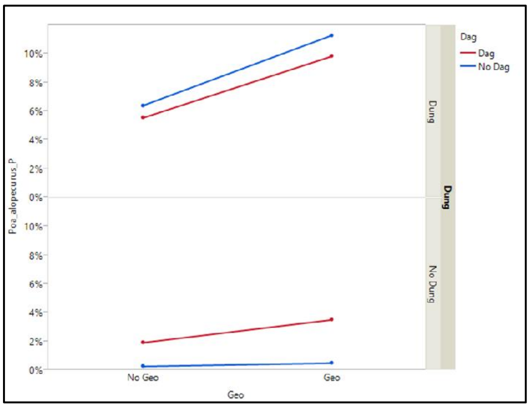
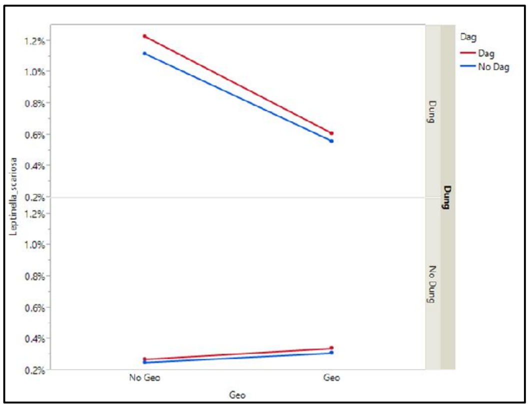


Fig.5d Interaction plots for Canopy of individual Seedmix species.

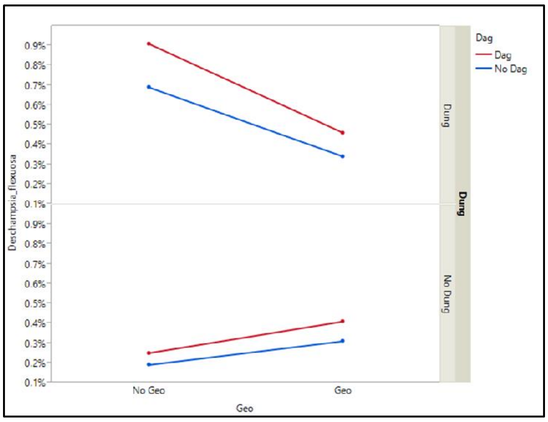
Poa alopecurus P



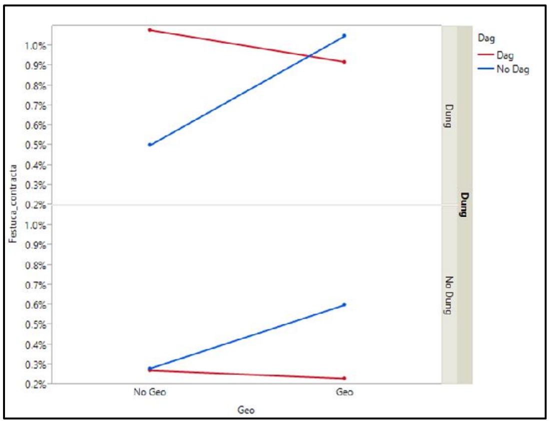
Leptinella scariosa



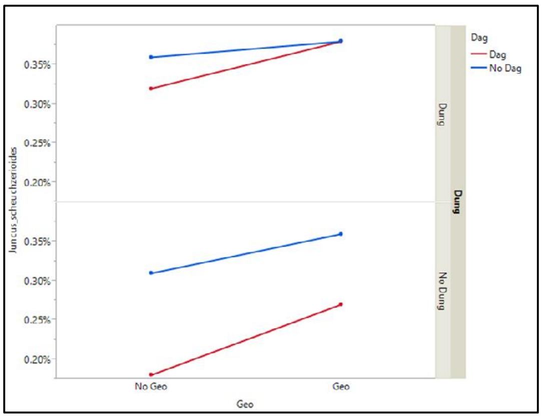
Deschampsia flexuosa



Festuca contracta



Juncus scheuchzerioides



Sediment

The cumulative plots of the sediment collection (Fig. 6) show a distinct seasonal pattern with fast accumulation (steep slopes) in the summer and slower accumulation (shallow slopes) in the winter. This is consistent for all soil types and regions.

Fig. 6a Cumulative plots of the sediment collection, points and smoothed line for each soil type.

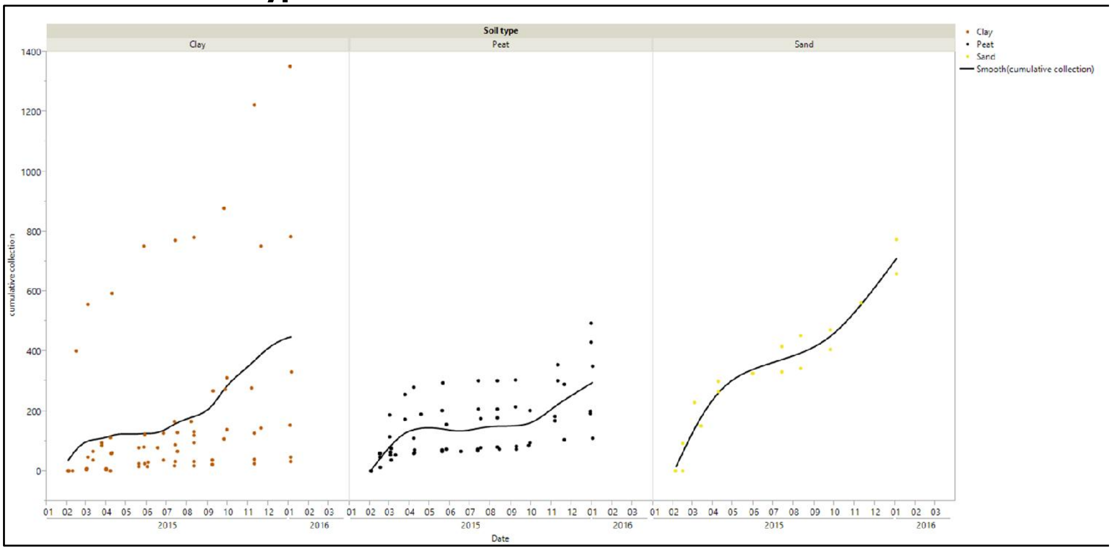


Fig. 6b Cumulative plots of the sediment collection, line for each site, grouped by soil type and coloured by region.

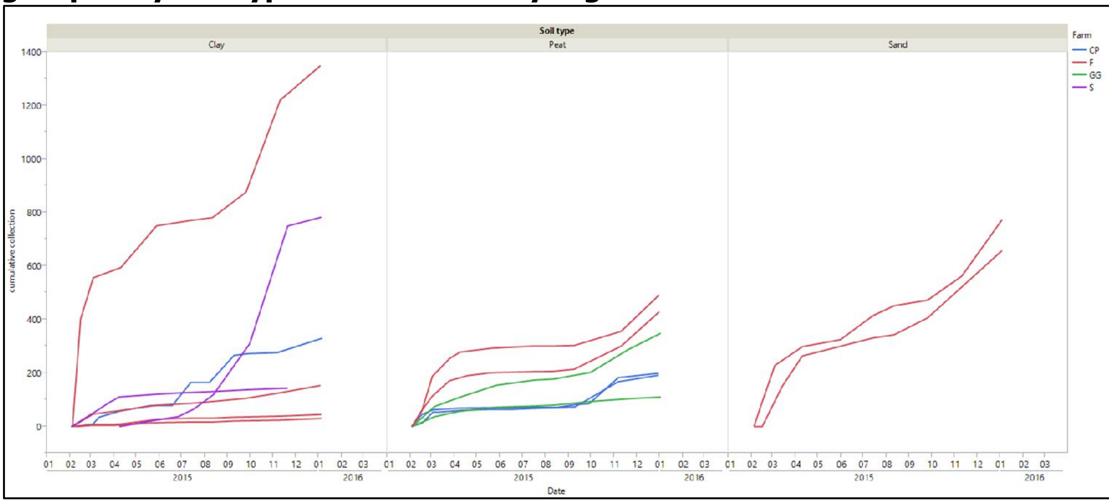


Fig. 6c Cumulative plots of the sediment collection, points and smoothed line for each region.

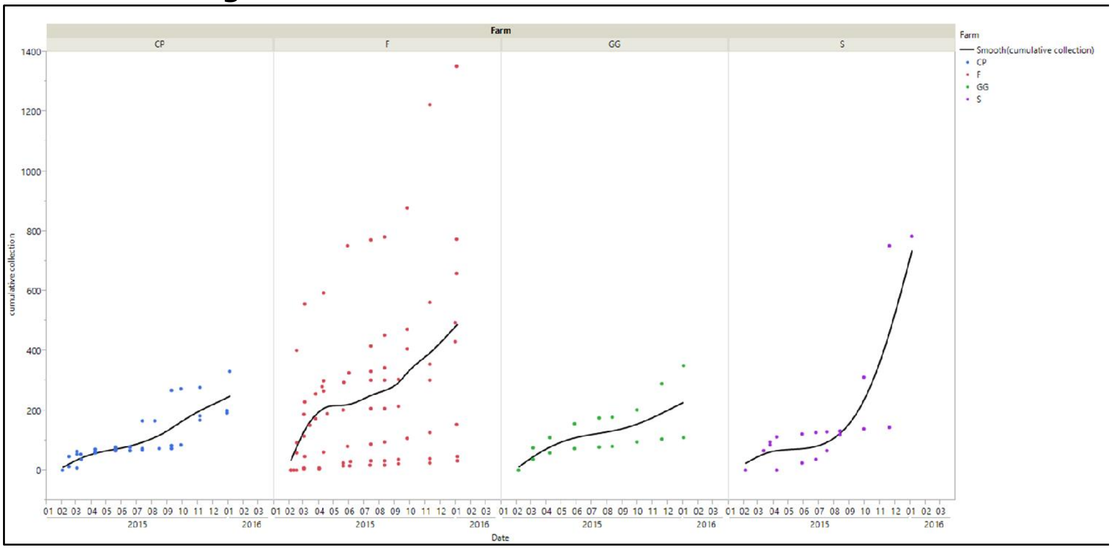
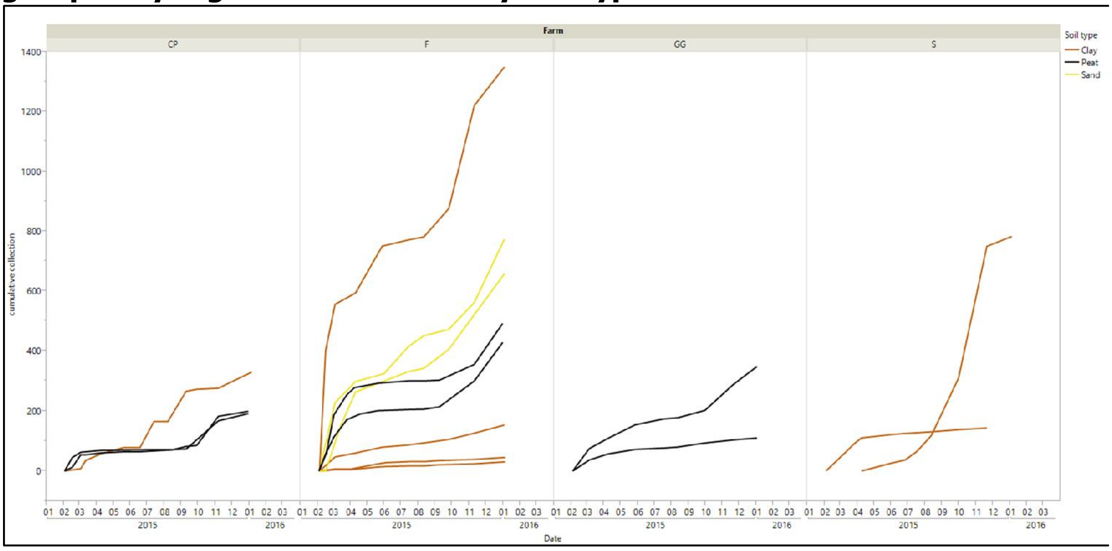


Fig. 6d Cumulative plots of the sediment collection, line for each site, grouped by region and coloured by soil type.



Multivariate Analysis

Subset 1 PCA

The loadings plot (Fig 7a) maps out the weights of the variables in the new components. The first component (x-axis) is the most important one and explains the most variability. Canopy cover, height and seedmix species are the key measures that contribute the most to this first component. These measures are close to each other and so are very correlated. Bareground is diagonally opposite to canopy cover and so the two variables are negatively correlated with each other.

The second component (y-axis) has temperature and moisture as the key measures. They are diagonally opposite to each other and so are negatively correlated.

The individual seedmix species are all on the right hand side of the loadings plot so they are correlated with the total canopy, height and the number of seedmix species. Non-seedmix species are to the left of the seedmix species and more central on the x-axis and therefore less correlated with the first component.

The scores plot (Figs 7b and 7c) maps out the responses of the plots in the new components. Two plots that are close to each other have similar profiles across the original measures whilst those that are far apart have diverse profiles. In Figure 7b we can see that plots with the same treatment are closer together with the no seed and geotextile plots on the left of the graph and the plots receiving all treatments and the dung and dag treatment combination on the right of the graph. Plots with high scores on the x-axis as seen in the loadings plot relates to more canopy cover, higher height of plants and more seedmix species.

In Figure 7c we can see that plots with the same soil type are closer together on the y-axis with plots on sand to the top of the graph, plots on clay in the middle and plots on peat at the bottom. Plots with high scores on the y-axis as seen in the loadings plot relates to higher temperatures and less moisture. Therefore, peat plots are damper and cooler whilst sand plots are warmer and dryer.

Fig. 7a PCA Loadings plot, Subset 1,

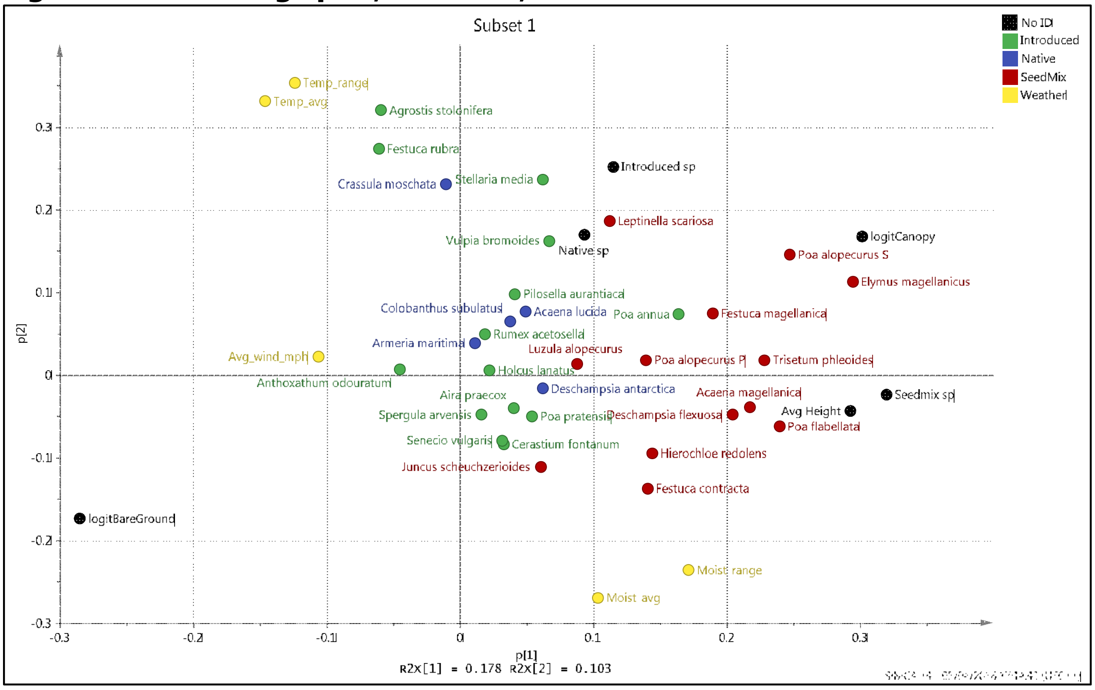


Fig. 7b PCA Scores plot, Subset 1, coloured and labelled by treatment.

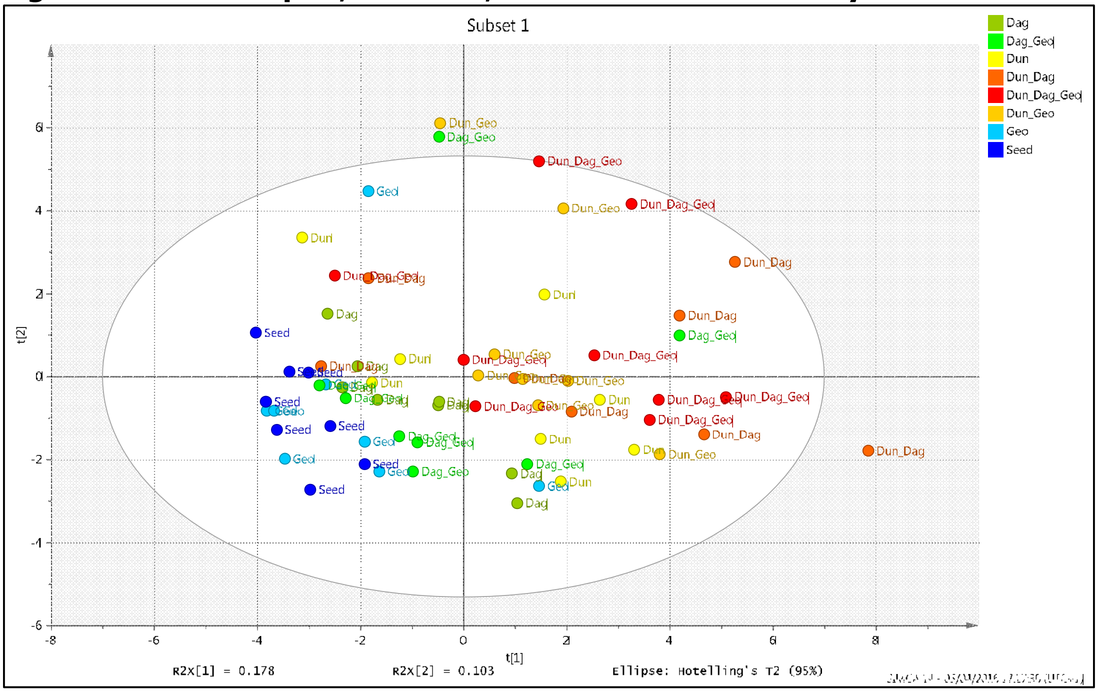
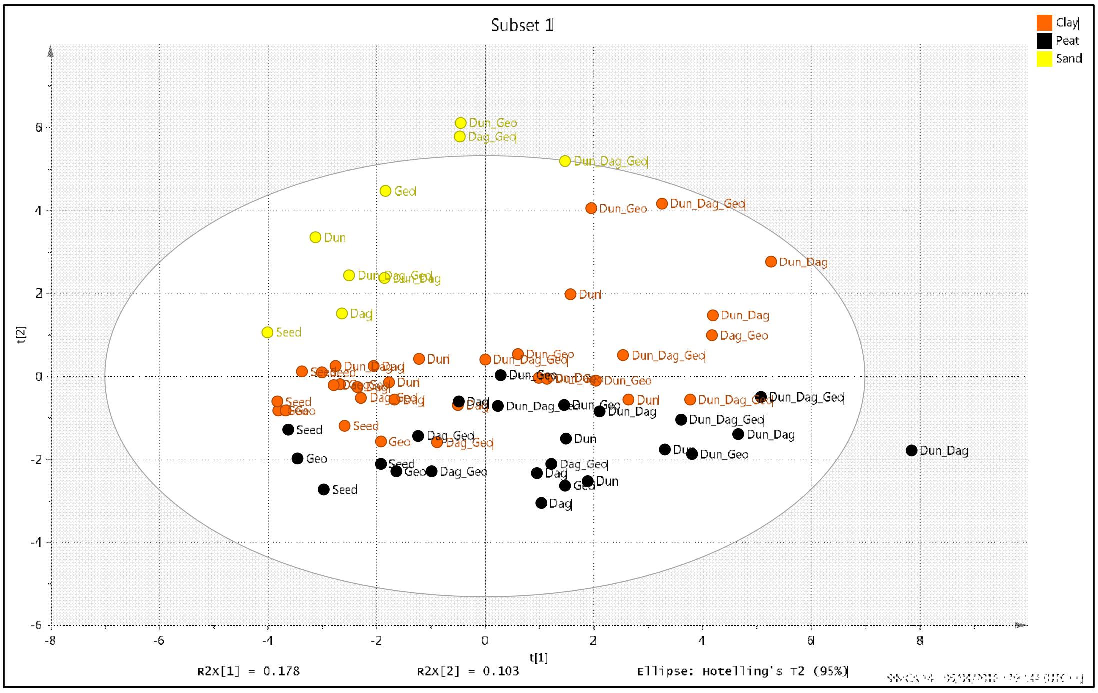


Fig. 7c PCA Scores plot, Subset 1, coloured by soil type and labelled by treatment.



Subset 1 PLS

Applying PLS to subset 1 a very similar picture is observed to the PCA. Loadings plots now include design features (treatments, soils and regions) as points. The treatments (dung, dags and geotextiles) have two points on the graph, presence and absence of the treatment, they lie equidistant on opposite sides of the origin. The further away from the origin the bigger the effect.

The first component (x-axis) in the loadings plot (Fig 8a) is the most important dimension and explains the most variability. Canopy, height, seedmix species and bareground are the key measures that contribute the most to this first component. The second component (y-axis) has temperature and moisture as the key measures. They are diagonally opposite to each other and so are negatively correlated.

In the scores plot (Fig. 8b) we can see that plots with the same treatment are closer together, with the no seed and geotextile plots on the left of the graph and the plots receiving all treatments and the dung.dag treatment combination on the right of the graph. This is a very similar pattern to the scores plot in the PCA.

Fig. 8a PLS Loadings plot, PC1 vs PC2, Subset 1,

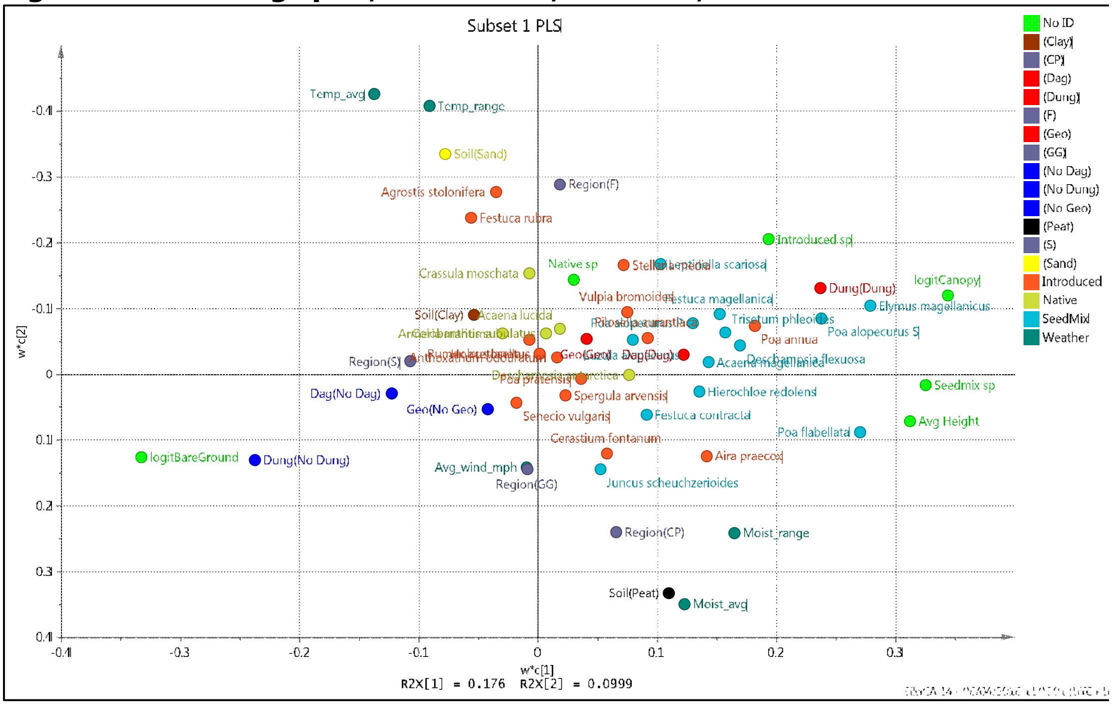
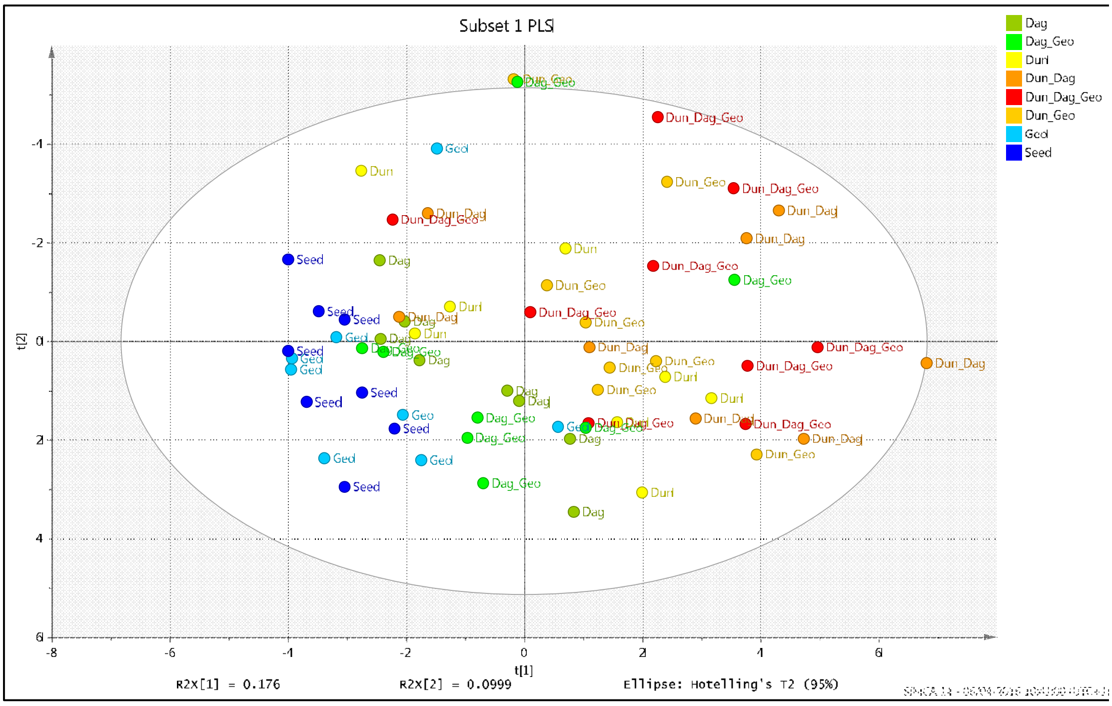


Fig. 8b PLS Scores plot, PC1 vs PC2, coloured and labelled by treatment, Subset 1.



Looking at the first principal component (PC1 on the x axis) in isolation we can interpret in more detail (Fig. 9a). Canopy, height, seedmix species and bareground are the key measures that contribute the most to this first component. However, temperature and moisture also contribute but to a lesser extent. Higher moisture and lower temperatures correlate with higher canopy cover, higher maximum height and a greater number of seedmix species.

Of the treatments, dung contributes the most to the higher levels of PC1, with dags contributing less, and geotextiles contributing only a small amount. The number of introduced species is also increased by these treatments; however, the number of native species not in the seedmix was not related to PC1 and any of the treatments. The canopy cover for all individual species in the seedmix are positively correlated with this first component, with *Elymus magellanicus, Poa flabellata* and *Poa alopecurus* (sand form) particularly strongly correlated. Many, but not all, introduced species are also positively correlated with the first component. The most strongly correlated species include: *Poa annua, Aira praecox* and *Pilosella aurantiaca*.

Peat plots appear to do better with treatment than clay and sand plots.

Fig. 9a PLS Loadings plot, PC1, Subset 1. First component (y axis) the x axis is arbitrary.

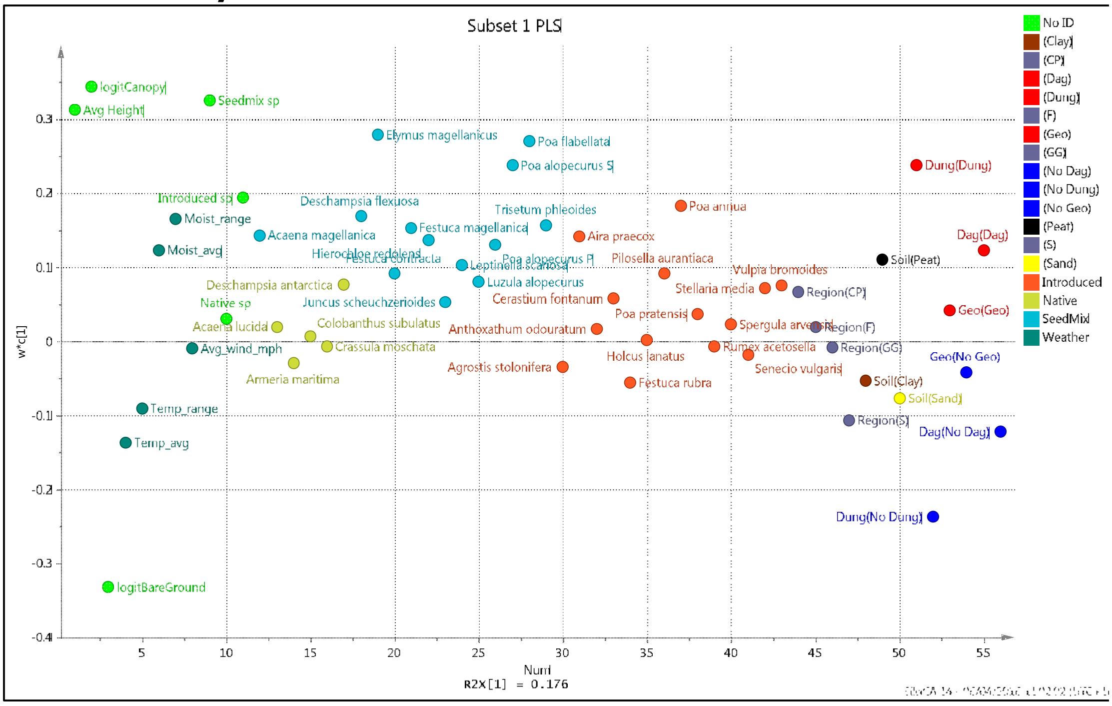
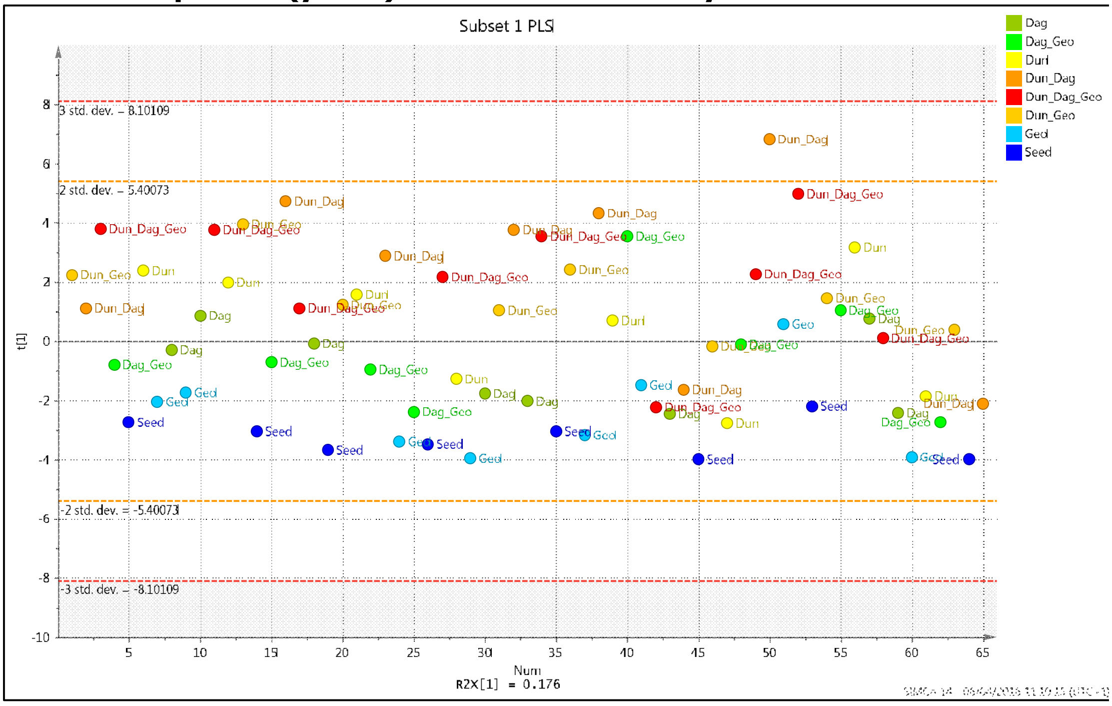


Fig. 9b PLS Scores plot, PC1, coloured and labelled by treatment, Subset 1. First component (y axis) the x axis is arbitrary.



The PLS modelling identifies a third important component. Plotting the loadings of the second and third components (Fig. 10a) we can see that the second component distinguishes between the peat and sand soil types whilst the third component distinguishes the clay soil type from the other two soils. Moisture and temperature are the key measures which distinguish the peat from the sand and clay. They also distinguish the Fitzroy region from other regions, as many of the clay plots at Fitzroy (bottom right of Fig 10b), as well as all the sand plots at Fitzroy are among the driest and warmest of all the plots.

The PLS modelling also suggest that establishment success of some species was related to soil type and region.

Of the seedmix species, *Festuca magellanica* does relatively better on clay, *Leptinella scariosa* does relatively better on sand and *Festuca contracta* does relatively better on peat.

Of the introduced species, *Agrostis stolonifera* and *Festuca rubra* do relatively better on sand, but the sand plots are specific to Fitzroy. *Aira praecox* does well on the peat, and *Cerastium fontanum* does well on peat plots at Cape Pembroke. However, as soil type and region are partially confounded with each other, it is difficult to draw clear-cut conclusions.

Fig. 10a PLS Loadings plot, PC2 vs PC3, Subset 1

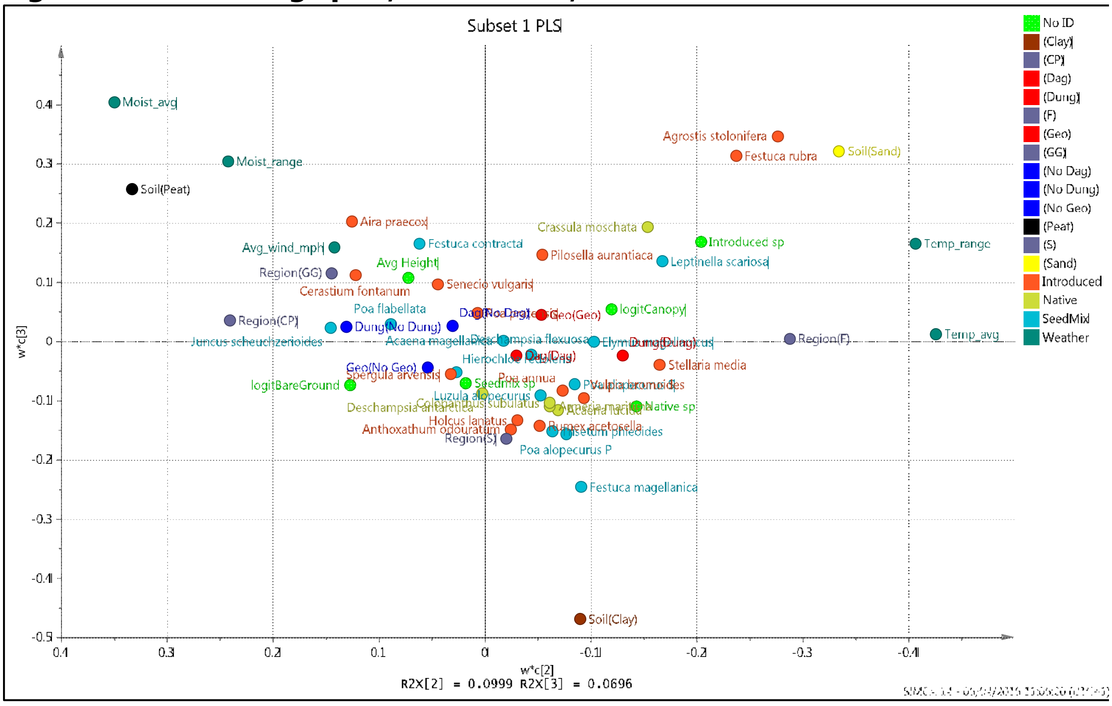
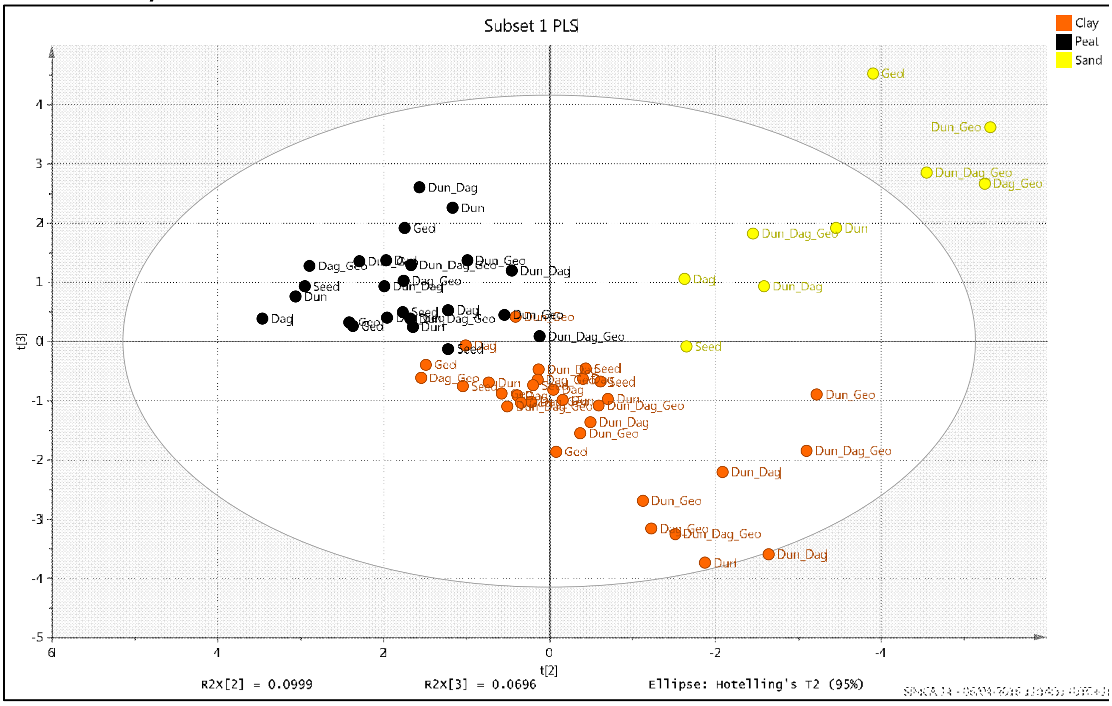
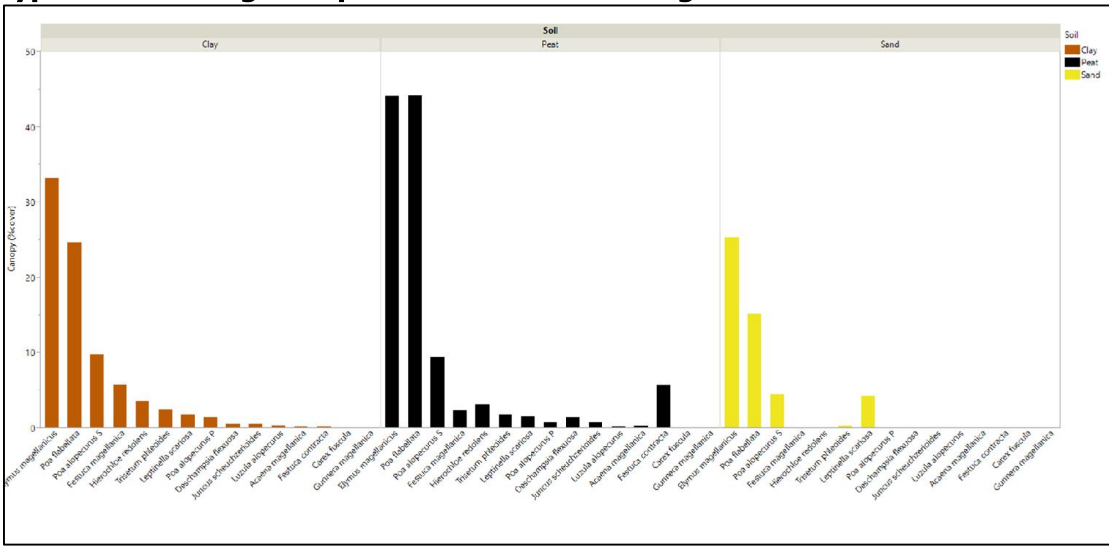


Fig. 10b PLS Scores plot, PC2 vs PC3, coloured by soil type, labelled by treatment, Subset 1



Profiles of seedmix species in the canopy (%cover) for each soil type is presented in Figure 11. *Elymus magellanicus, Poa flabellata* and *Poa alopecurus* (sand ecotype) dominate all restored plots across all three soil types with other native sown species typically having less than 5% cover. The relative strengths of *Festuca magellanica* on clay, *Leptinella scariosa* on sand and *Festuca contracta* on peat can also be seen in Figure 11 confirming the conclusions from the PLS analysis (Figure 10a).

Fig. 11 Profiles of seedmix species in the canopy (%cover) for each soil type. Raw averages of plots that received dung.



Subset 2 PCA

Subset 2 includes the weather measures and all species that flowered in at least one plot. Species measures are binary, 1=flowered, 0=not flowered/not present. PCAs are not as sensitive to binary measures as they are to continuous measures, so this PCA produced less clear results than the PCA performed on subset 1.

The first component (x-axis) in the loadings plot (Fig 12a) is the most important dimension and explains most of the variation. Temperature and moisture are the key measures that contribute most to this first component. The second component (y-axis) has a selection of species as the key measures.

In Figure 12b we can see that plots with the same treatment are closer together on the y-axis, with the no seed and geotextile plots at the centre top of the graph. The plots receiving all treatments, and those with the dung and dag treatment combination are at the periphery of the graph. In Figure 12c we can see that the plots are grouped by soil type again with peat on the left, sand on the right and clay at the centre and bottom.

A group of seedmix species (*Poa flabellata, Festuca contracta, Deschampsia flexuosa* and *Hierochloe redolens*) lie on the left of the loadings plot (Fig 11a). These tend to flower in peat plots that are moist and cool and have had dung and dag treatments. A second group of seedmix species (*Trisetum phleoides, Elymus magellanicus, Festuca magellanica* and *Poa alopecurus* (sand form)) and a group of introduced species (*Aira praecox, Vulpia bromoides, Poa annua* and *Stellaria media*) group in the centre bottom of the graph. These species tend to flower in two sets of plots: plots on clay soils; and those peat plots with mid-range temperatures and moistures, which were treated with dung in combination with either dags or geotextiles.

Fig. 12a PCA Loadings plot, Subset 2

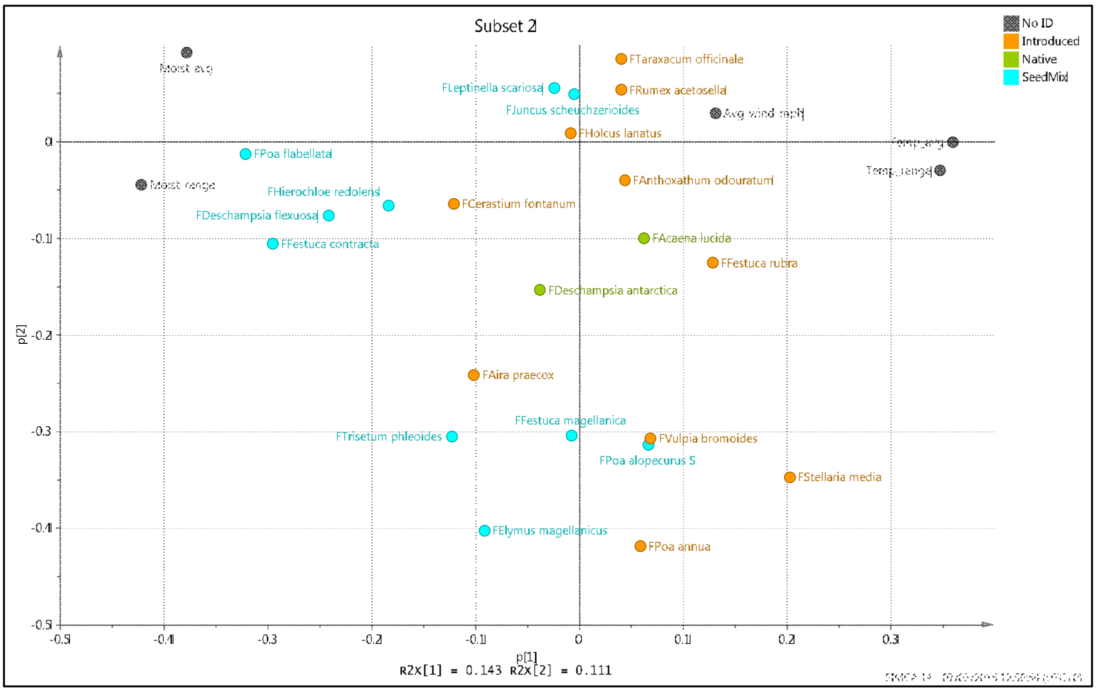


Fig. 12b PCA Scores plot, coloured by and labelled by treatment, Subset 2

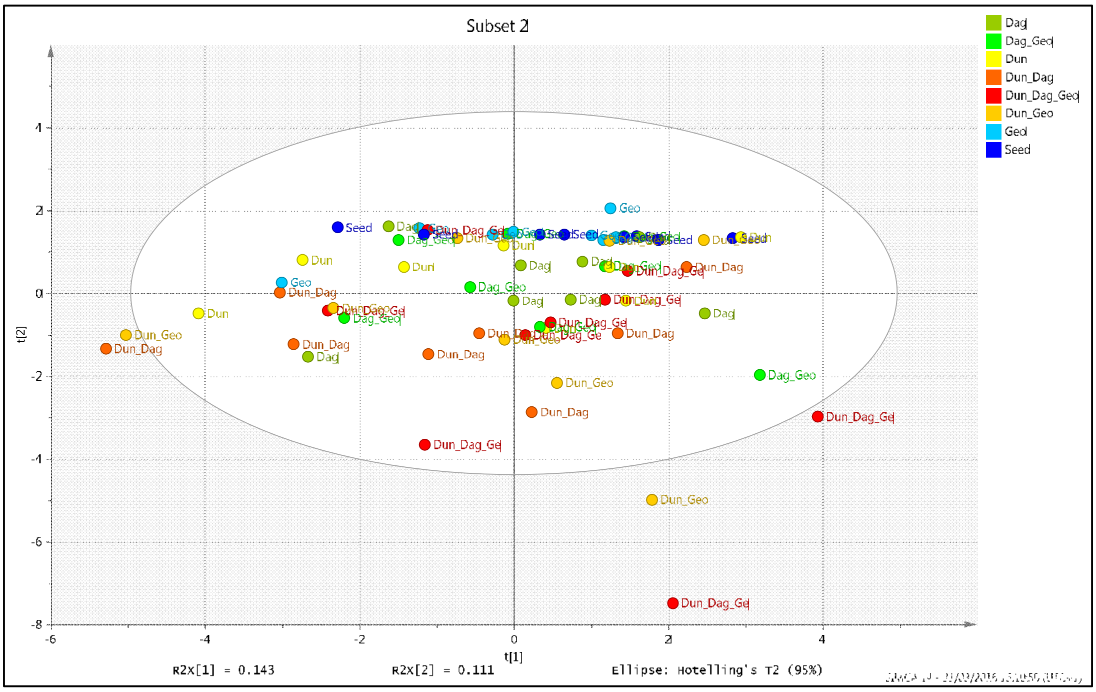
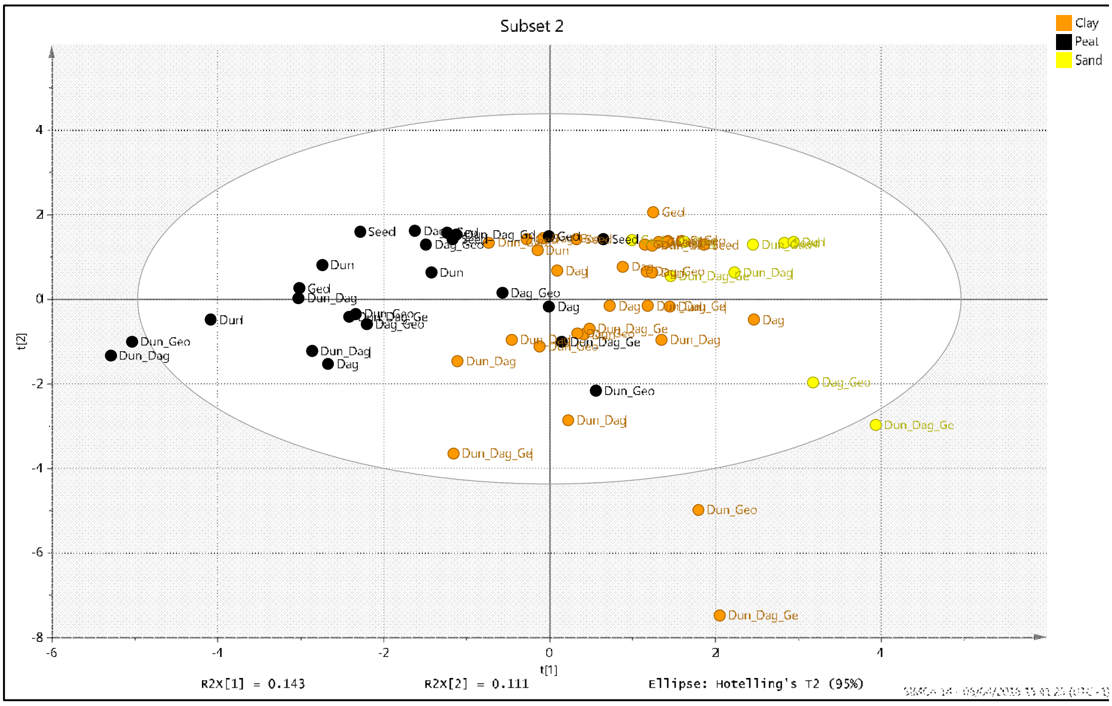


Fig. 12c PCA Scores plot, coloured by soil type, labelled by treatment, Subset 2



Conclusions

The main conclusions are:

1. The use of dung, dags and geotextiles significantly increases the canopy cover, total plant biomass, maximum plant height and number of sown native species present across all soil types.
2. Of these three treatments, dung is the most effective, followed by dags. The use of geotextiles is the least effective.
3. Combinations of treatments can be more effective than single treatments but the size of the effect is not simply the addition of the individual effects.
4. Treatments may have differential effects on sand than on clay and peat. Dung may be less effective on sand and geotextiles more effective. However, these conclusions are suggested tentatively due to limited replication of sand sites in the experiment design and the possible impact of flooding of the sand sites.
5. Three sown native species dominant plant cover across all soil types: *Elymus magellanicus, Poa flabellata* and *Poa alopecurus* (sand ecotype). However, other native species have higher cover on specific soil types, namely *Festuca magellanica* on clay, *Leptinella scariosa* on sand and *Festuca contracta* on peat.
6. All treatments increase the number of introduced species, with dung having the largest impact. However, the presence of specific species appears to be related to site and region. As dung and dags were all sourced from the same location, it is more likely that the increase with treatment is related to creating better conditions for introduced species to grow than species being introduced with the treatment.
7. Application of native seed mixture alone ie. without treatment did not increase plant cover when compared to plots without seed mixture or treatment.
8. Flowering was found to be species specific and related to environmental conditions (moisture and temperature) as well as soil type.

References

1. Patterson, H.D. & Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika*, **58**, 545-554.
2. Genstat Version 18.1.0.17005 (VSN International Ltd.)
3. SIMCA Version 14.0.0.1359 (Umetrics AB)
4. Randall D. Tobias, SAS Institute Inc., Cary, NC, USA. An Introduction to Partial Least Squares Regression http://www.ats.ucla.edu/stat/sas/library/pls.pdf
5. Charles Zaiontz, Basic Concepts of Logistic Regression. http://www.real-statistics.com/logistic-regression/basic-concepts-logistic-regression/

Appendix – Experimental Design

Within site plot layout:

A	B	C	D
E	F	G	H

Key

Factorial Design treatments
no seed control with all treatments
no seed control with no treatments

Region

CP	Cape Pembroke
GG	Goose Green
F	Fitzroy
S	Saledero

	Site	Region	Soil	Plot	Treatment	Dung	Geo	Dag	Seed	Block
1	1	CP	Clay	A	Dun_Geo	Dung	Geo	No Dag	Seed	1
2	1	CP	Clay	B	Dun_Dag	Dung	No Geo	Dag	Seed	1
3	1	CP	Clay	C	Dun_Dag_Geo	Dung	Geo	Dag	Seed	1
4	1	CP	Clay	E	Dag_Geo	No Dung	Geo	Dag	Seed	1
5	2	CP	Clay	A	Trt_Con	Dung	Geo	Dag	No Seed	1
6	2	CP	Clay	B	No_Seed	No Dung	No Geo	No Dag	No Seed	1
7	2	CP	Clay	C	Seed	No Dung	No Geo	No Dag	Seed	1
8	2	CP	Clay	E	Dun	Dung	No Geo	No Dag	Seed	1
9	2	CP	Clay	F	Geo	No Dung	Geo	No Dag	Seed	1
10	2	CP	Clay	H	Dag	No Dung	No Geo	Dag	Seed	1
11	3	CP	Peat	C	Geo	No Dung	Geo	No Dag	Seed	2
12	3	CP	Peat	D	Dag	No Dung	No Geo	Dag	Seed	2
13	3	CP	Peat	E	No_Seed	No Dung	No Geo	No Dag	No Seed	2
14	3	CP	Peat	F	Dun_Dag_Geo	Dung	Geo	Dag	Seed	2
15	3	CP	Peat	G	Dun	Dung	No Geo	No Dag	Seed	2
16	3	CP	Peat	H	Trt_Con	Dung	Geo	Dag	No Seed	2
17	4	CP	Peat	A	Dun_Geo	Dung	Geo	No Dag	Seed	2
18	4	CP	Peat	B	Seed	No Dung	No Geo	No Dag	Seed	2
19	4	CP	Peat	C	Dag_Geo	No Dung	Geo	Dag	Seed	2
20	4	CP	Peat	F	Dun_Dag	Dung	No Geo	Dag	Seed	2
21	4	CP	Peat	G	Trt_Con	Dung	Geo	Dag	No Seed	2
22	4	CP	Peat	H	No_Seed	No Dung	No Geo	No Dag	No Seed	2
23	7	GG	Peat	A	No_Seed	No Dung	No Geo	No Dag	No Seed	4
24	7	GG	Peat	B	Dun_Dag_Geo	Dung	Geo	Dag	Seed	4
25	7	GG	Peat	C	Dag	No Dung	No Geo	Dag	Seed	4
26	7	GG	Peat	E	Seed	No Dung	No Geo	No Dag	Seed	4

27	7	GG	Peat	G	Trt_Con	Dung	Geo	Dag	No Seed	4
28	7	GG	Peat	H	Dun_Geo	Dung	Geo	No Dag	Seed	4
29	8	GG	Peat	A	Dun	Dung	No Geo	No Dag	Seed	4
30	8	GG	Peat	C	No_Seed	No Dung	No Geo	No Dag	No Seed	4
31	8	GG	Peat	D	Dag_Geo	No Dung	Geo	Dag	Seed	4
32	8	GG	Peat	E	Dun_Dag	Dung	No Geo	Dag	Seed	4
33	8	GG	Peat	F	Geo	No Dung	Geo	No Dag	Seed	4
34	8	GG	Peat	G	Trt_Con	Dung	Geo	Dag	No Seed	4
35	9	F	Clay	A	Trt_Con	Dung	Geo	Dag	No Seed	5
36	9	F	Clay	B	Dag_Geo	No Dung	Geo	Dag	Seed	5
37	9	F	Clay	C	No_Seed	No Dung	No Geo	No Dag	No Seed	5
38	9	F	Clay	F	Seed	No Dung	No Geo	No Dag	Seed	5
39	9	F	Clay	G	Dun_Dag_Geo	Dung	Geo	Dag	Seed	5
40	9	F	Clay	H	Dun	Dung	No Geo	No Dag	Seed	5
41	10	F	Clay	A	Geo	No Dung	Geo	No Dag	Seed	5
42	10	F	Clay	B	Dag	No Dung	No Geo	Dag	Seed	5
43	10	F	Clay	C	Dun_Geo	Dung	Geo	No Dag	Seed	5
44	10	F	Clay	E	Trt_Con	Dung	Geo	Dag	No Seed	5
45	10	F	Clay	G	Dun_Dag	Dung	No Geo	Dag	Seed	5
46	10	F	Clay	H	No_Seed	No Dung	No Geo	No Dag	No Seed	5
47	11	F	Clay	A	Dag	No Dung	No Geo	Dag	Seed	6
48	11	F	Clay	C	No_Seed	No Dung	No Geo	No Dag	No Seed	6
49	11	F	Clay	D	Dun_Dag_Geo	Dung	Geo	Dag	Seed	6
50	11	F	Clay	E	Trt_Con	Dung	Geo	Dag	No Seed	6
51	11	F	Clay	F	Seed	No Dung	No Geo	No Dag	Seed	6
52	11	F	Clay	H	Dun_Geo	Dung	Geo	No Dag	Seed	6
53	12	F	Clay	B	No_Seed	No Dung	No Geo	No Dag	No Seed	6
54	12	F	Clay	C	Trt_Con	Dung	Geo	Dag	No Seed	6
55	12	F	Clay	D	Geo	No Dung	Geo	No Dag	Seed	6
56	12	F	Clay	E	Dun_Dag	Dung	No Geo	Dag	Seed	6
57	12	F	Clay	G	Dun	Dung	No Geo	No Dag	Seed	6
58	12	F	Clay	H	Dag_Geo	No Dung	Geo	Dag	Seed	6
59	13	F	Sand	B	Geo	No Dung	Geo	No Dag	Seed	7
60	13	F	Sand	D	Trt_Con	Dung	Geo	Dag	No Seed	7
61	13	F	Sand	E	Dun_Dag_Geo	Dung	Geo	Dag	Seed	7
62	13	F	Sand	F	No_Seed	No Dung	No Geo	No Dag	No Seed	7
63	13	F	Sand	G	Dag	No Dung	No Geo	Dag	Seed	7
64	14	F	Sand	B	Dun_Dag	Dung	No Geo	Dag	Seed	7
65	14	F	Sand	C	Seed	No Dung	No Geo	No Dag	Seed	7
66	14	F	Sand	D	Dun_Geo	Dung	Geo	No Dag	Seed	7
67	14	F	Sand	F	Dun	Dung	No Geo	No Dag	Seed	7
68	14	F	Sand	G	Dag_Geo	No Dung	Geo	Dag	Seed	7
69	14	F	Sand	H	Dun_Dag_Geo	Dung	Geo	Dag	Seed	7
70	15	F	Peat	A	Dun_Dag	Dung	No Geo	Dag	Seed	8
71	15	F	Peat	B	Geo	No Dung	Geo	No Dag	Seed	8
72	15	F	Peat	E	No_Seed	No Dung	No Geo	No Dag	No Seed	8
73	15	F	Peat	F	Dun_Dag_Geo	Dung	Geo	Dag	Seed	8
74	15	F	Peat	G	Seed	No Dung	No Geo	No Dag	Seed	8

75	15	F	Peat	H	Trt_Con	Dung	Geo	Dag	No Seed	8
76	16	F	Peat	B	Dun_Geo	Dung	Geo	No Dag	Seed	8
77	16	F	Peat	D	Dag_Geo	No Dung	Geo	Dag	Seed	8
78	16	F	Peat	E	No_Seed	No Dung	No Geo	No Dag	No Seed	8
79	16	F	Peat	F	Dun	Dung	No Geo	No Dag	Seed	8
80	16	F	Peat	G	Dag	No Dung	No Geo	Dag	Seed	8
81	16	F	Peat	H	Trt_Con	Dung	Geo	Dag	No Seed	8
82	17	S	Clay	A	Dun_Dag_Geo	Dung	Geo	Dag	Seed	9
83	17	S	Clay	B	Dag	No Dung	No Geo	Dag	Seed	9
84	17	S	Clay	C	No_Seed	No Dung	No Geo	No Dag	No Seed	9
85	17	S	Clay	D	Geo	No Dung	Geo	No Dag	Seed	9
86	17	S	Clay	F	Dun	Dung	No Geo	No Dag	Seed	9
87	17	S	Clay	G	Trt_Con	Dung	Geo	Dag	No Seed	9
88	18	S	Clay	B	Dag_Geo	No Dung	Geo	Dag	Seed	9
89	18	S	Clay	C	No_Seed	No Dung	No Geo	No Dag	No Seed	9
90	18	S	Clay	D	Dun_Geo	Dung	Geo	No Dag	Seed	9
91	18	S	Clay	E	Seed	No Dung	No Geo	No Dag	Seed	9
92	18	S	Clay	F	Trt_Con	Dung	Geo	Dag	No Seed	9
93	18	S	Clay	G	Dun_Dag	Dung	No Geo	Dag	Seed	9