THE EFFECTS OF NESTING GULLS ON THE SHINGLE VEGETATION AT ORFORD NESS SUFFOLK

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SUMMARY

It has been shown that nesting gulls may alter the vegetation surrounding their nesting colonies through eutrophication and disturbance.
These effects were studied on the vegetated shingle ridges at Orford Ness
Available nutrient concentrations were shown to be significantly higher at abandoned nest sites for Phosphate, ammonium and magnesium. Over-winter leaching was shown to significantly reduce available nutrient concentrations at abandoned nest-sites.
Species cover for *Silene uniflora*, *Arrhenatherum elatius*, *Homalothecium lutescens* and the lichen genus *cladonia* did not differ significantly between abandoned nest and non nest sites.
Differences in turnover of available nitrogen between abandoned nest and non-nest sites were not significant in terms of plant nutrition.
Glasshouse experiments suggest *Silene uniflora*, and *Arrhenatherum elatius* Respond well to increases in available nitrogen and a combination of nitrogen and phosphorus. These results remain true when the species are grown in the presence of each other. This Suggests competition between the two species is not a limiting factor.
Gull disturbance through aggressive interactions on the vegetated shingle ridges appears minimal
The finding from this study suggest that the gulls and plants can co-exist without extensive alteration to the shingle ecosystem.

Introduction

Gulls may potentially affect the vegetation around their nesting colonies through nutrient enrichment and disturbance (Evans & Davy 2000; Gillham 1956; Sobey & Kenworth 1979). In nutrient-poor shingle, habitats such processes would be likely to be detrimental to plant biodiversity. Local and less competitive species may suffer due to dominance by more nutrient demanding species (Tidswell 1993; Harding & Crewe 1994).

Shingle has been classified as sediment larger than sand (< 2 mm) and smaller than boulders (> 200 mm) (Fuller 1989). Britain's coastline incorporates approximately 900 km of pure shingle, much of which is unvegetated. Vegetation is usually restricted to large successive areas of shingle such as stable ridges and spits, which offer greater protection from wave action (Chapman 1976). On a global scale shingle beaches represent a rare ecosystem that is susceptible to many forms of damage both natural and anthropogenic (Fuller & Randall 1988)

Orford Ness is a National Trust site which encompasses Europe's largest vegetated spit. The site has been described as one of the best examples of vegetated shingle in Europe (Fuller & Randall 1988). The spit is formed almost entirely of flint, deposited over time by constructive wave action through the process of long-shore drift (Carr 1972). The greatest influence on spit formation has come from storm waves which throw the shingle over the top of the beach crest, leaving it protected from the reach of ordinary wave action (Green & Mc Gregor 1989). This process of deposition over time can lead to the formation of a complex system of stable, parallel ridges and swails (Carr 1972). The wave action has the effect of naturally sorting the shingle with finer particles being deposited on the ridge crests, and larger shingle being deposited in the swails. (Fuller 1987). This natural gradation of shingle plays an important role in plant establishment (Scott 1963). The higher percentage of fines found on the ridge tops has been related to several factors which assist in plant colonisation (Fuller & Randall 1988). Shingle has a high porosity with low water

retention; fine particles have been shown to increase water retention in shingle and so reduce drought effects. A high percentage of fine particles also restricts the depth which seeds can be washed down the column (Davy, Willis & Beerling 2000). The combination of these factors leads to higher germination and survival rates for plants growing in shingle with a high percentage of fine fraction (Fuller 1987)

Orford Ness offers an outstanding representation of a shingle ecosystem and a rare example of shingle plant succession. The diversity of vegetation ranges from early strandline pioneers including *Lathyrus japonicus* and *Crambe maritima* , also long lived perennials such as *Silene uniflora* and *R*umex *crispus* on the more stable ridges. The grass *Arrhenatherum elatius* can be found throughout the site from the seaward ridges through to the older ridges. The succession progressing finally to the second largest area of shingle acid-heath in Britain (Fuller & Randall 1988).

The Heath is an important site for many moss and lichen species, with an abundance of normally epiphytic lichen species such as *Parmelia caperata* and *Evernia prunastre* (Harding & Crewe 1994). By far the most predominant lichen genus found on the heath is *Cladonia*. (Fuller & Randall 1988).

Orford Ness is also a Internationally important ornithological site, with a large mixed gull colony. In recent history, gulls first colonised the site over thirty years ago. Nesting birds increasing progressively, with a dramatic increase since 1995 (Fig. 1)#(Cormack 1999). There were approximately 23,000 breeding pairs of Lesser black-backed gulls (*Larus fuscus*) and 5100 breeding pairs of herring gulls (*Larus argentatus*) recorded on site in 1999. The gulls build their nest's in depressions in the shingle from local vegetation and line the final structure with moss and lichen thalli (Tidswell 1993).

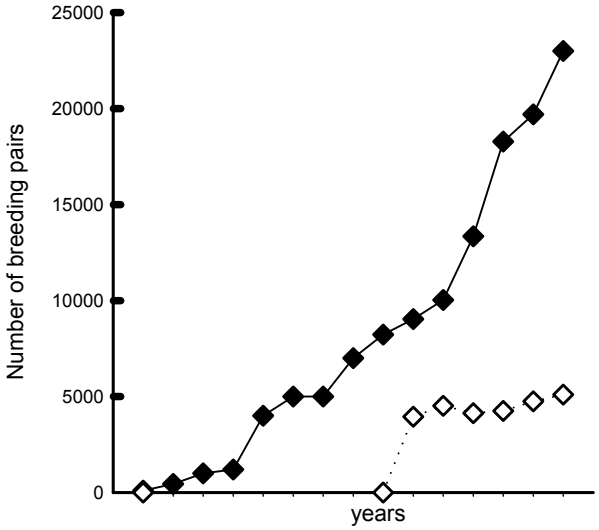


Figure 1. Increase in gull population over the past 30 years
filled symbol, *Larus fuscus;* open symbol, *Larus argentatus*

Shingle plants have adapted to survive adverse environmental conditions such as drought, temperature extremes, and low mineral nutrient regimes. Any large input of nutrients especially when coupled with disturbance could have detrimental effects on the slow-growing shingle species.

The aim of the research was to investigate whether nesting gulls might affect the vegetation surrounding their nests, through the processes of:

- Nutrient enrichment through the deposition of food scraps and guano;
- Disturbance through territorial disputes such as boundary clashes
- The large input of organic nesting material onto the shingle ridges.

2.0.0 Methods section

2.1.0 Field Work

2.1.1 Study area

The study site was situated at the southern Southwest end of the Ness and fell in the then English Nature owned National Nature Reserve (TM 418-471 to TM 424-474). The area consisted of well defined vegetated shingle ridges with the dominant ridge species consisting of *Silene uniflora* and *Arrhenatherum elatius*.
The area was selected after a visual survey of the Ness in the first week of March 1999. The site selected had well defined vegetated shingle ridges with co-existing *Silene uniflora* and *Arrhenatherum elatius*. The ridge system showed minimal disturbance and was home to a large, mixed, nesting gull population

2.1.2 Sampling sites

Sampling was carried out during the last week in May 1999 and the third week in January 2000, along two parallel ridges. The samples consisted of twenty-five nest sites, which were paired with twenty-five vegetated non-nest sites. Non nest sites were selected within 7 m either side of the nest site using random numbers. At each sample site a $1m^2$ quadrat was placed, at nest sites the quadrat was placed around the centre of the nest.

2.1.3 Vegetation Sampling

A 1m^2 quadrat area was chosen for the vegetation survey as it incorporated the main vegetation belt along the top of the ridge and also gave an accurate measure of percentage cover by evening out the patch effects in vegetation as described by Bullock (1996).

Species were identified with percentage cover determined, for each quadrat. The two ridge plant species *Silene uniflora* and *Arrhenatherum elatius* were dominant along with lichens of the genus *Cladonia*. The lichen species recorded at genus level because of the difficulty in identification, with most species needing careful microscopic examination to confirm their identity (Kershaw & Alvin 1966). The moss *Homalothecium lutescens* was also recorded.
Species cover was visually estimated and recorded as a percentage cover for each of the five plant species.

2.1.4 Substrate Survey

At each quadrat four samples of shingle were removed. These consisted of one 25 cm $\times$ 25 cm^2 sample which was taken from each corner of the quadrat giving a total shingle sample of 100 cm x 100 cm^2. Each sample was carefully removed using a standard gardening trowel to try to maintain the shingle profile. Each sample was then passed through a series of soil sieves (31.5 mm, 16 mm, 8 mm, 4 mm) the weight of each fraction was recorded in the field. The fine fraction (<4 mm) was sealed in marked plastic bags and taken back to the lab for further analysis.

The sample sites were then refilled with the graded shingle, the smallest shingle fraction was placed on top to reduce the effects of disturbance. While on site the soil samples were boxed and stored in a refrigerator at 2 ^{o}C in darkness.

2.2.0 Laboratory analysis

2.2.1 Sample storage

All soil samples were removed from Orford Ness and stored in a cold room at 2 ^{o}C in complete darkness before analysis. Laboratory analysis of the fine fraction was undertaken on one day within a two week period from collection.

2.2.2 Soil sample & solution preparation

The four soil samples from each quadrat were weighed using a Sartorius bench scale (tolerance $\pm$ 1 mg) and mixed to give one bulked sample per quadrat
Soil nutrient analysis was carried out using an aqueous soil extract. The solution was obtained by shaking 25 g of fine fraction with 125 ml of ultra pure Milli-Q water (Grade 1, conductivity less than 17.8 μOhm). The samples were placed on a rotary shaker for 60 minutes after which they were centrifuged at 6000 rpm for 10 minutes using a M.S.E minor bench centrifuge. The aqueous solution was then removed using a 20 ml syringe and filtered through a 0.2 micron Sartorius syringe filter. The filtered solution was then placed into a 25 ml screw top plastic vial and frozen at -24^{o}C until analysis.

2.2.3 Anion analysis

Anion analysis was carried out using an Ion-Chromatograph (DIONEX-DX-100).
The filtered samples were analysed for Chloride, Nitrate, Phosphate and Sulphate. Standards were made for each of the anions analysed and full details of these and of sample preparation are given in Appendix 2 after Schutten (1998).

2.2.4 Analysis of Ammonium

Analysis of ammonium was carried out using an ammonium-specific electrode. Milli-volt readings for each filtered aqueous sample were converted to concentrations (mg l^{-1}), using a calibration curve constructed with 7 ammonium standards with IM lithium acetate added. Full details of analysis and sample preparation are given in appendix 2 after Schutten (1996)

2.2.5 Atomic Absorption Spectrometry

A Pye Unicam SP-191 atomic absorption spectrometer with air-acetylene flame was used to analyse the concentration of Calcium (Ca), Magnesium (Mg), and Manganese (Mn) in the filtered aqueous samples. A series of known standards for the three nutrients were run to give calibration curves for each nutrient analysed. The resulting absorbance readings for each solution were converted into concentrations (mg l^{-1}) with the use of the calibration curves

2.2.6 Soil Mineralization

The turnover of soil nitrogen was analysed using the fine fraction from the May nest and non nest-site samples. The fine fraction from each sample quadrat was divided into six 25 g sub sample groups each consisting of fifty soil samples (table 1).
The samples from five of the sub sample groups were placed in 125 ml marked glass flasks and sealed with NescoFilm moisture proof self sealing tape. The remaining group of fifty sub samples were sealed in marked polythene bags. All samples were refrigerated overnight in darkness at 2^{o} C. Group A was extracted as described in section 2.2.2 immediately . Groups B-E were placed in a Gallenkamp illuminated incubator in darkness at a temperature of 20^{o} C for varying time periods (as shown in table 1). Group F was transported onto site and buried on the original ridge at a depth of 25 cm for a period of four weeks. After each incubation period all samples were extracted using the methods described in section 2.2.3 and analyzed for ammonium and nitrate as described in sections 2.2.4 and 2.2.5 respectively

Sample sub groups	Nest site samples	Non Nest-site samples	Incubation period
A	25	25	No incubation
B	25	25	1 week
C	25	25	2 weeks
D	25	25	3 weeks
E	25	25	4 weeks
F	25	25	4 weeks on site

Table 1. Sample sub groups Incubation periods.

2.3.0 Glasshouse Experiments

2.3.1 Plant nutrient experiments

The effects of additions of phosphate and nitrogen on the two dominant species *S. uniflora* and *A. elatius*, were Investigated using a glasshouse experiment. Each species was grown separately under a series of nutrient treatments. A combination of the two species were also grown in combination to look for competitive interactions. The concentrations of phosphate and nitrogen used as treatment in the experiments (Table 2) were set at values higher than those found in the field, (Fig. 4 b, Fig. 5 a). This was to compensate for reductions in the field sample concentrations due to leaching and other losses in the field.

A four-block treatment design (Table. 2) was used for each experiment.

Each block consisted of 9, 10-inch labelled flowerpots, filled with a 1:4 mixture of grade 21 Arnold sand and 10 mm washed shingle. Each pot was sown with 10 seeds on the surface, these were covered with 2-4 mm of the sand. For the combined species pots 10 seeds from each of the species were sown.
The pots for each experiment were distributed randomly within the four blocks and placed in a greenhouse at 25/15 ^{o}C. The greenhouse was illuminated with 400 W high-pressure sodium lamps 0800-2400 (16h day). After initial germination the seedlings were thinned to give an equal number of six plants in each block. The plants were allowed to grow for a three months with each pot receiving a weekly nutrient treatment (Table. 2) diluted in 250 ml of ultra pure Milli-Q water (Grade 1, conductivity less than 17.8 μOhm).
After three months the plants were carefully harvested, placed in marked paper bags and dried in a force draft hot air oven for 48 hours at 70 ^{o}C. The root, shoot and total biomass for each plant was recorded, and biomass per pot was used for analysis.
The seeds from each species were collected from the study site during the 2nd week of July 1999. *Silene uniflora* seeds were placed in moist 21 grade Arnold sand and stored in darkness at 2 ^{o}C six weeks prior to the experiment to break dormancy. *Arrhenatherum elatius* seeds did not require cold treatment and were stored in darkness at room temperature.

Treatment Block	**Number Of pots**	**P (NaH_2PO_4)**	**N (NH_4NO_3)**
1	9	0	0
2	9	5 mM	0
3	9	0	10 mM
4	9	5 mM	10 mM

Table 2. Nutrient treatments for blocks, in 250ml aqueous solution

2.4.0 Data Analysis

2.4.1 Independent t-test

Differences between nest and non nest-sites for particle distribution, species cover and nutrient concentrations were statistically analysed with SPSS software, using independent t-tests

2.4.2 One way ANOVA

Differences in soil Mineralization results and plant nutrient experiments were statistically analysed with SPSS release 9, software, using a one way ANOVA with Tukey post-hoc test.

3.0 Results

3.1.0 Particle size distribution

The data for particle size distribution was summarised as bar charts and displayed in figures 2 and 3. Significant differences ($P < 0.05$) between sites were analysed using an analysis of variance with Turkey post hoc test, the results from which are displayed in tables 3 and 4.

3.1.1 May 1999 samples

The distribution of particle sizes between the sites can be seen in Figure 2. The results indicate there were no significant ($P < 0.05$) differences in particle size distribution between abandoned nest and non nest sites as shown in table 3

Particle size	t	df	Nest (g)	Nest (s.e)	Non Nest (g)	Non Nest (s.e)	Sig. (P)
> 31.5 mm	-0.807	48	4406.8	± 327.50	4848.8	± 439.03	0.424
> 16 mm	-0.506	48	40726	± 1712.8	41748	± 1072.4	0.615
> 08 mm	-0.414	48	23602	± 1228.3	24202	± 768.44	0.681
> 04 mm	-1.722	48	2940.6	± 245.68	3910	± 506.48	0.091
04 mm	-0.833	48	466.74	± 33.564	516.66	± 49.625	0.409

Table 3. comparisons between mays abandoned nest and non-nest sites of the shingle particle distribution. Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; Independent t-tests)

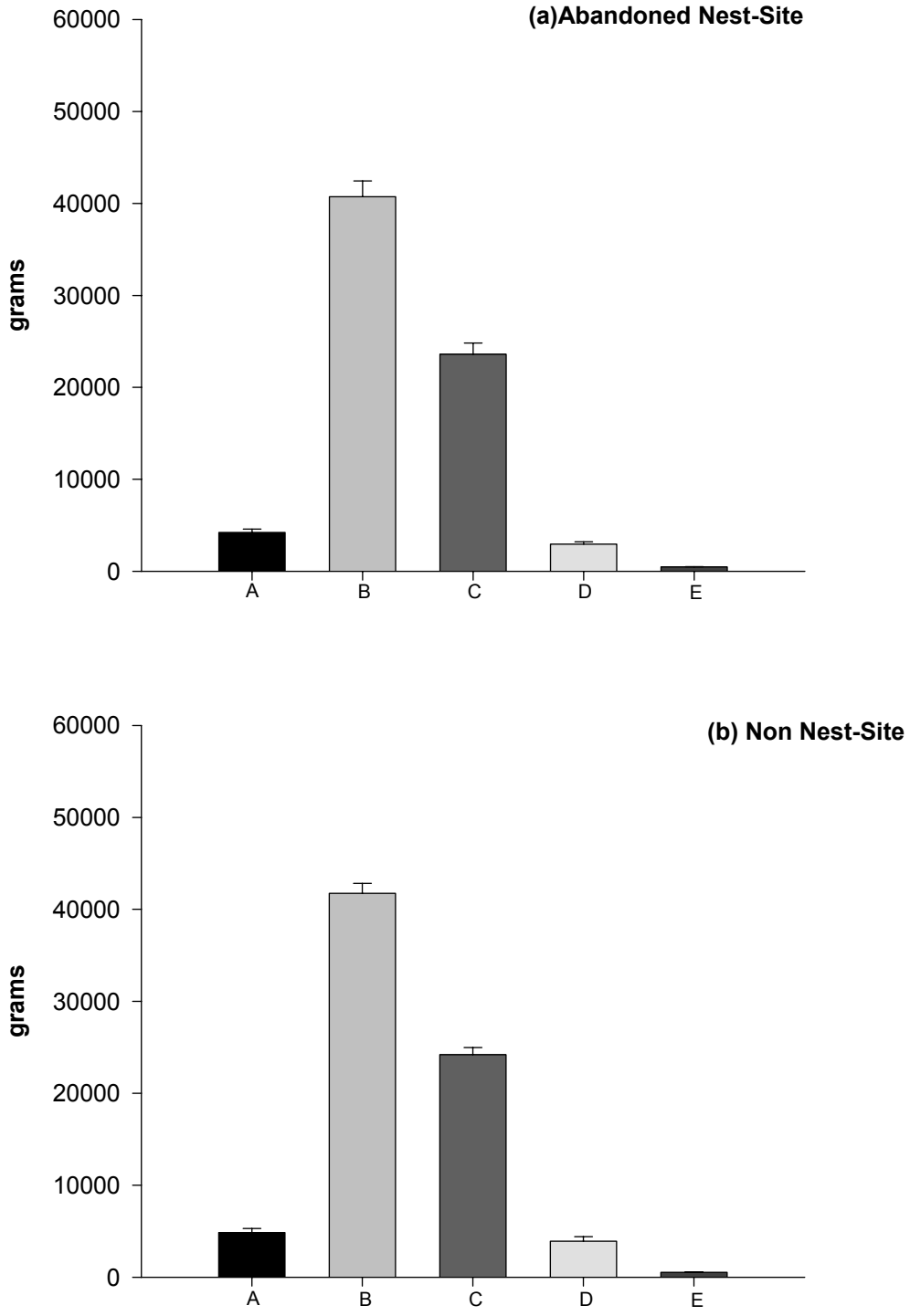


Figure 2. Comparison of shingle particle distribution per $1m^2$ quadrat of the May samples (a) abandoned nest-sites (b) non nest-sites. Bars represent A, > 32 mm, B, > 16 mm, C, > 8 mm, D, > 4 mm, E, 4 mm. Error bars represent one standard error (n =25)

3.1.2 January 2000 samples

The distribution of particle sizes between the sites can be seen in figure 3. The results indicate there were no significant ($P < 0.05$) difference in particle size distribution between abandoned nest and non nest sites as shown in table 4

Particle size	t	df	Nest (g)	Nest (s.e)	Non Nest (g)	Non Nest (s.e)	Sig. (P)
> 31.5 mm	.000	48	4214.96	± 339.62	4215.15	± 368.88	1.00
> 16 mm	.480	48	47680	± 881.62	47088	± 862.89	0.63
> 08 mm	-.281	48	19232	± 788.34	19551.20	± 819.35	0.78
> 04 mm	-.907	48	2386.40	± 82.14	2566	± 180.29	0.36
04 mm	1.110	48	64.02	± 12.80	224.54	± 11.76	0.27

Table 4. comparisons between January abandoned nest and non-nest sites of the shingle particle distribution. Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; Independant t-tests)

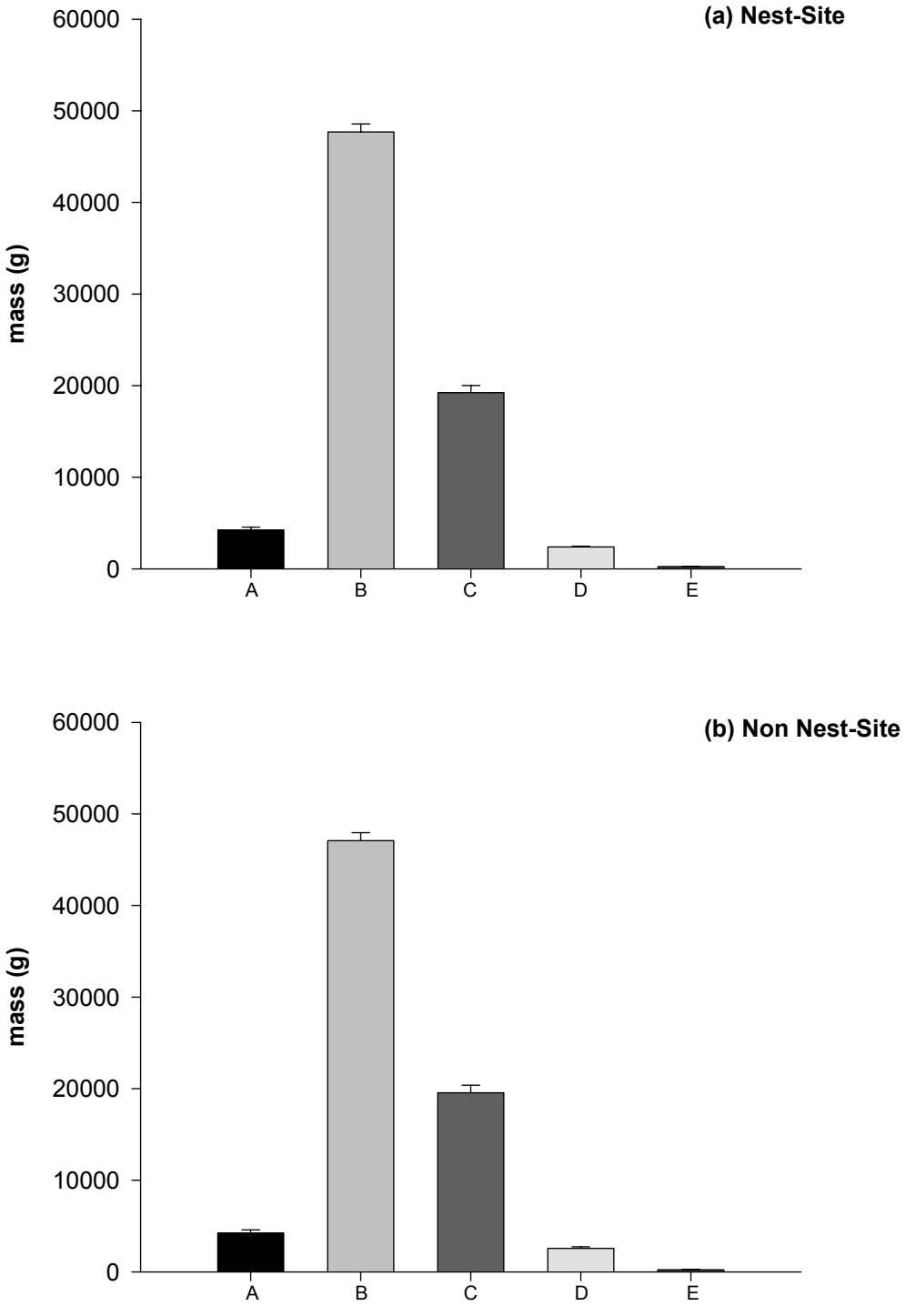


Figure 3. Comparison of shingle particle distribution per $1m^2$ quadrat of the January samples (a) abandoned nest-sites (b) non nest-sites. Bars represent A, > 32 mm, B, > 16 mm, C, > 8 mm, D, > 4 mm, E, 4 mm. Error bars represent one standard error (n =25)

3.2.0 Nutrient availability

The data for nutrient availability was summarised as bar charts and displayed in figures 4, 5, 6, and 7. Significant differences between nest and non nest sites was analysed using an Independent t-test, the results from which are displayed in tables 5, and 6.

Differences in abandoned nest site nutrient availability between the May and January samples were compared to look at nutrient availability over time. The data was summarised as bar charts and displayed in figures 8, and 9. Significant differences ($P < 0.05$) between May and January nest sites was analysed using an Independent t-test, the results from which are displayed in table 7.

3.2.1 May 1999 samples

The results from the May samples as shown in figures 4, and 5, indicate that significant differences ($P < 0.05$) in nutrient availability as shown in table 5 exist between abandoned nest and non-nest sites. Chloride, magnesium and ammonium concentrations are each significantly higher at abandoned nest sites showing a P value of < 0.05, sulphate and phosphate being more abundant at abandoned nest sites with a P value of <0.01.

Soil Nutrients Mg l^{-1}	t	df	Nest (μ)	Nest (s.e)	Non Nest (μ)	Non Nest (s.e)	Sig. (P)
Chloride	1.880	48	25.416	$\pm$ 3.658	16.602	$\pm$ 1.143	0.02*
Nitrate	0.883	48	95.966	$\pm$ 7.589	79.728	$\pm$ 5.204	0.08
Phosphate	13.167	48	24.967	$\pm$ 3.665	10.368	$\pm$ 1.241	0.00**
Sulphate	6.400	48	14.819	$\pm$ 2.294	8.196	$\pm$ 0.766	0.00**
Magnesium	2.462	48	6.111	$\pm$ 0.862	3.989	$\pm$ 0.547	0.04*
Manganese	2.491	48	0.226	$\pm$ 0.087	0.137	$\pm$ 0.022	0.32
Calcium	1.814	48	3.552	$\pm$ 0.376	2.796	$\pm$ 0.231	0.09
Ammonium	2.468	48	0.083	$\pm$ 0.016	0.038	$\pm$ 0.006	0.02*

Table 5. Comparisons of nutrient availability (mg l^{-1} in aqueous solution) between abandoned nests and non-nest sites. Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; Independant t-tests).

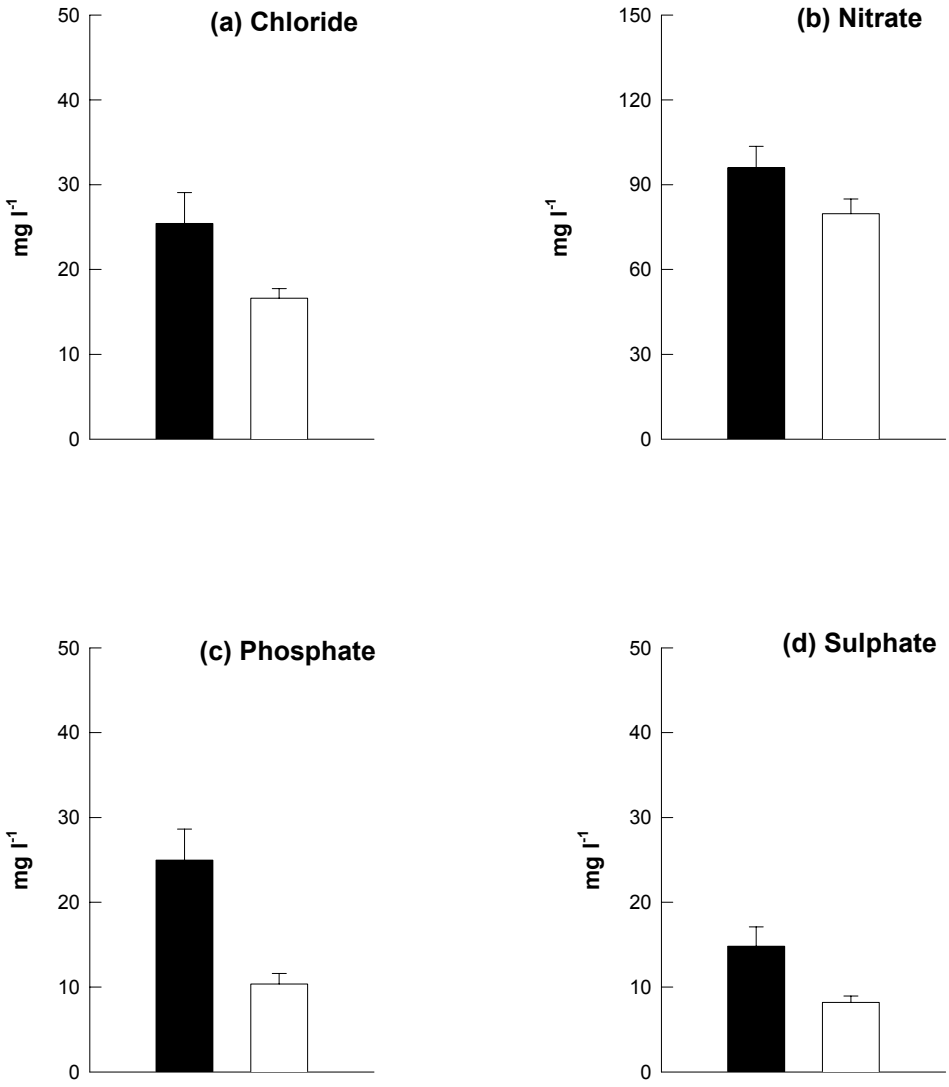


Figure 4. comparisons of nutrient availability (mg l^{-1} in aqueous extract) between May abandoned nest sites and non-nest sites: (a) Chloride, (b) Nitrate, (c) Phosphate (d) Sulphate. filled bars, abandoned nest; open bars, non-nest. Error bars represent one standard error (n = 25)

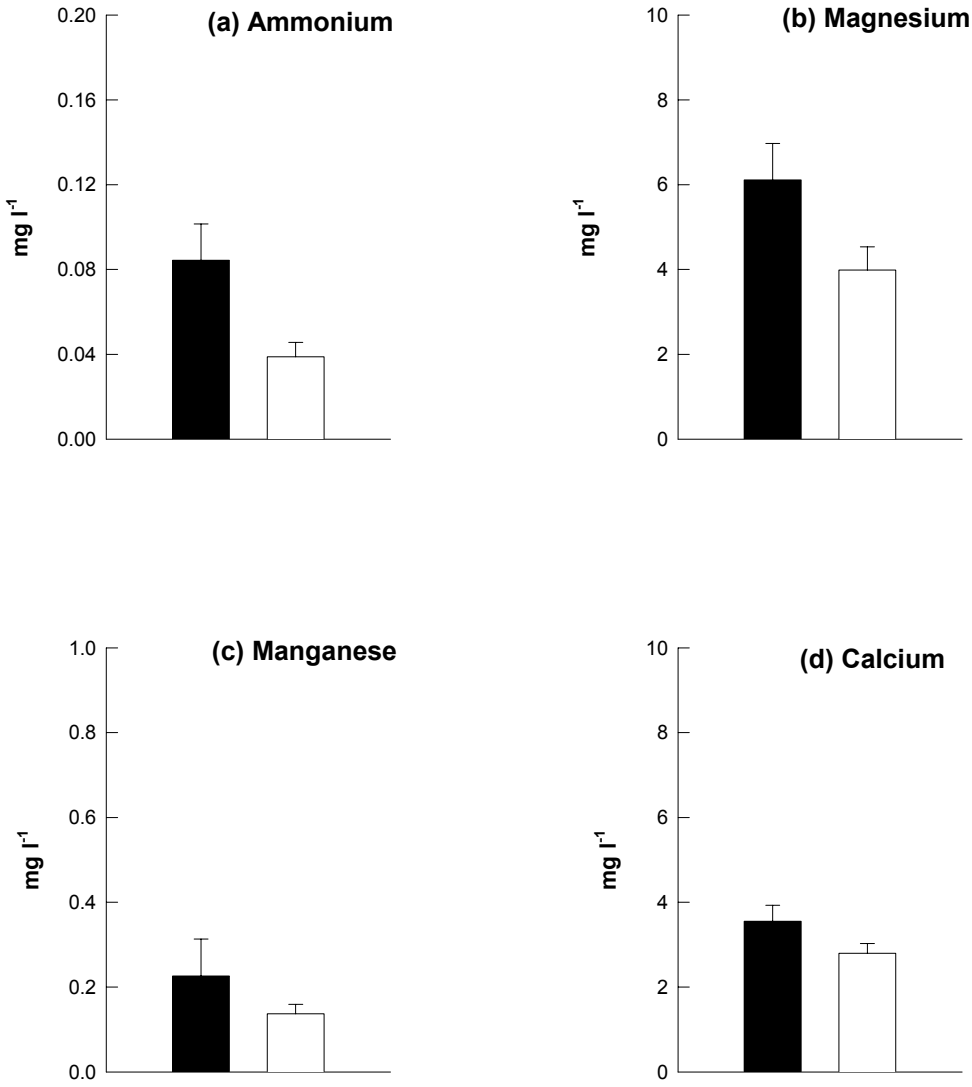


Figure 5. comparisons of nutrient availability (mg l^{-1} in aqueous extract) between May abandoned nest sites and non-nest sites: (a) Ammonium, (b) Magnesium, (c) Manganese (d) Calcium. filled bars, abandoned nest; open bars, non-nest. Error bars represent one standard error (n = 25)

3.2.2 January 2000 samples

The results from the January samples as shown in figures 6, and 7, indicate that significant differences ($P < 0.05$) in nutrient availability as shown in table 6 exist between abandoned nest and non-nest sites. Chloride and phosphate concentrations are each significantly higher at non-nest sites showing a P value of < 0.05, with magnesium and ammonium concentrations being more abundant at abandoned nest sites with a P value of <0.05.

Soil Nutrients Mg l^{-1}	t	df	Nest (μ)	Nest (s.e)	Non Nest (μ)	Non Nest (s.e)	Sig. (P)
Chloride	-2.283	48	1.850	$\pm$ 0.072	20.90	$\pm$ 0.071	0.02*
Nitrate	-1.511	48	11.569	$\pm$ 0.651	13.665	$\pm$ 1.225	0.13
Phosphate	-2.277	48	0.224	$\pm$ 0.012	0.274	$\pm$ 0.017	0.02*
Sulphate	-1.955	48	2.246	$\pm$ 0.070	2.438	$\pm$ 0.068	0.06
Magnesium	2.362	48	9.298	$\pm$ 2.091	4.186	$\pm$ 0.557	0.02*
Manganese	1.822	48	0.2925	$\pm$ 0.035	0.212	$\pm$ 0.025	0.07
Calcium	0.609	48	4.014	$\pm$ 0.454	3.654	$\pm$ 0.376	0.54
Ammonium	2.073	48	0.046	$\pm$ 0.005	0.033	$\pm$ 0.004	0.044*

Table 6. . Comparisons of nutrient availability (mg l^{-1} in aqueous solution) between abandoned nests and non-nest sites. Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; Independant t-tests).

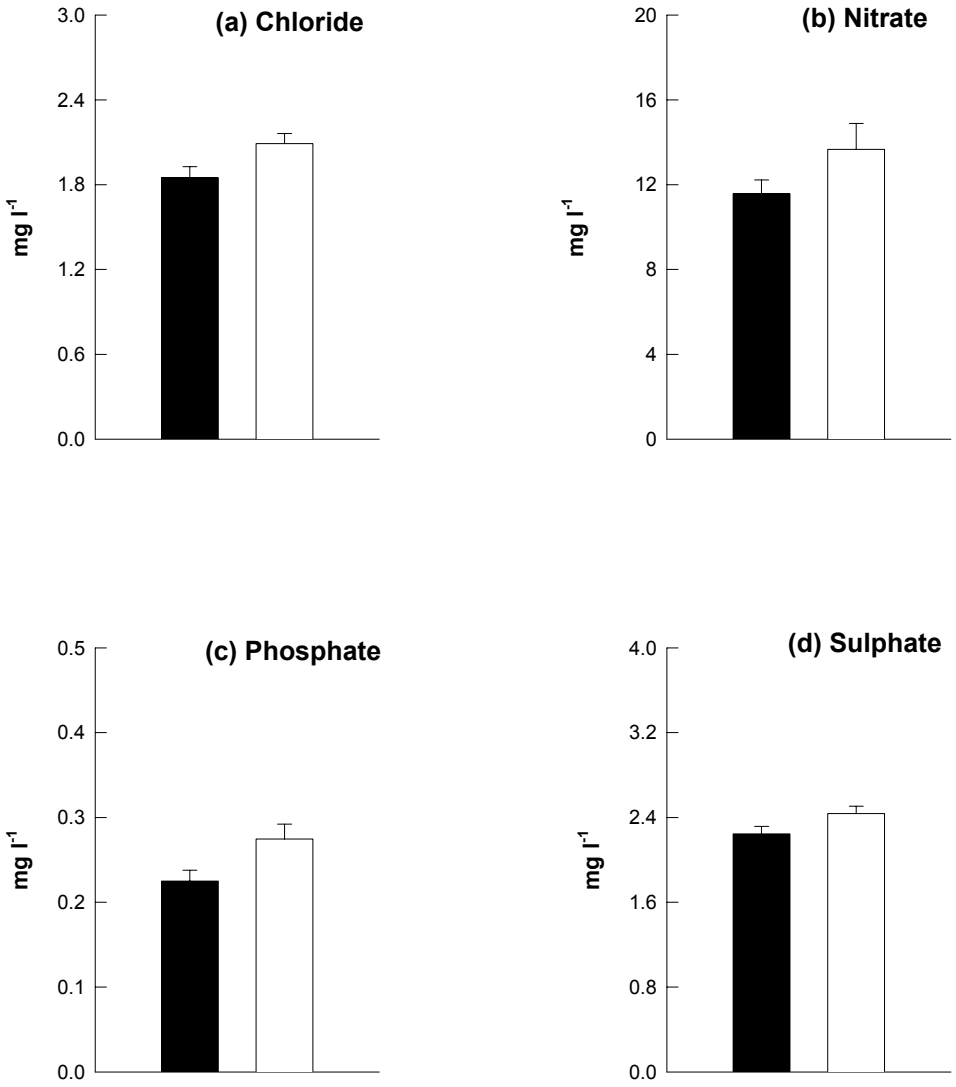


Figure 6. comparisons of nutrient availability (mg l^{-1} in aqueous extract) between January abandoned nest sites and non-nest sites: (a) Chloride, (b) Nitrate, (c) Phosphate (d) Sulphate. filled bars, abandoned nest; open bars, non-nest. Error bars represent one standard error (n = 25)

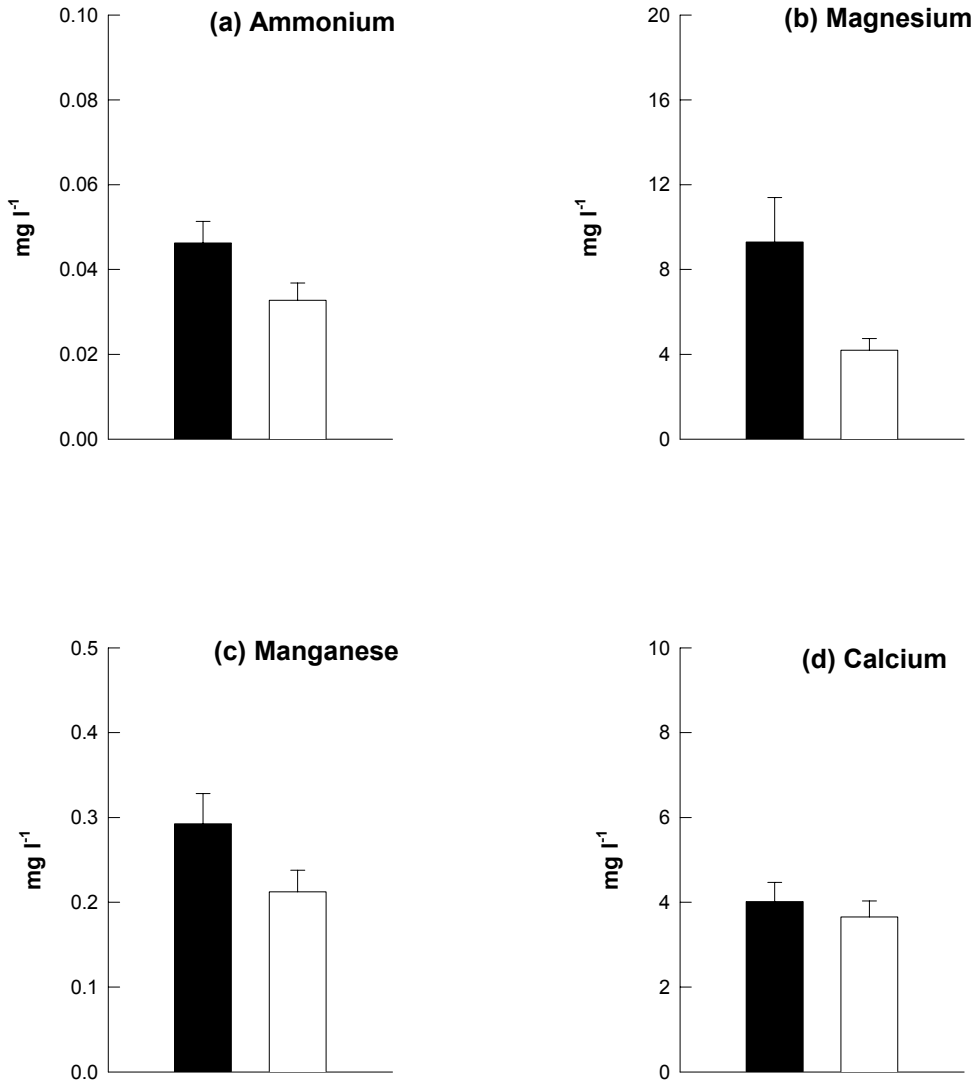


Figure 7. comparisons of nutrient availability (mg l^{-1} in aqueous extract) between January abandoned nest sites and non-nest sites: (a) Ammonium, (b) Magnesium, (c) Manganese (d) Calcium. filled bars, abandoned nest; open bars, non-nest. Error bars represent one standard error (n = 25)

3.2.3 comparison of May 1999 and Jan 2000 samples

The results from the combined abandoned nest site samples as shown in figures 8, and 9, indicate that significant differences ($P < 0.05$) in nutrient availability as shown in table 7 exist between May and January abandoned nest sites. Ammonium concentrations are significantly higher at Mays abandoned nest sites showing a P value of < 0.05, with chloride, nitrate, phosphate and sulphate concentrations being more abundant at Mays abandoned nest sites with a P value of <0.01.

Soil Nutrients Mg l^{-1}	t	df	Nest (M) (μ)	Nest (M) (s.e)	Non Nest (J) (μ)	Non Nest (J) (s.e)	Sig. (P)
Chloride	6.440	48	25.416	$\pm$ 3.658	1.850	$\pm$ 0.077	0.00**
Nitrate	11.080	48	95.966	$\pm$ 7.589	11.569	$\pm$ 0.651	0.00**
Phosphate	6.750	48	24.967	$\pm$ 3.665	0.224	$\pm$ 0.012	0.00**
Sulphate	5.477	48	14.819	$\pm$ 2.294	2.246	$\pm$ 0.070	0.00**
Magnesium	-1.408	48	6.111	$\pm$ 0.862	9.298	$\pm$ 2.091	0.165
Manganese	0.705	48	0.226	$\pm$ 0.087	0.292	$\pm$ 0.035	0.484
Calcium	-0.784	48	3.552	$\pm$ 0.376	4.014	$\pm$ 0.454	0.437
Ammonium	2.137	48	0.084	$\pm$ 0.017	0.046	$\pm$ 0.005	0.037*

Table 7. Comparisons of nutrient availability (mg l^{-1} in aqueous solution) between may and January abandoned nest sites. Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; Independant t-tests).

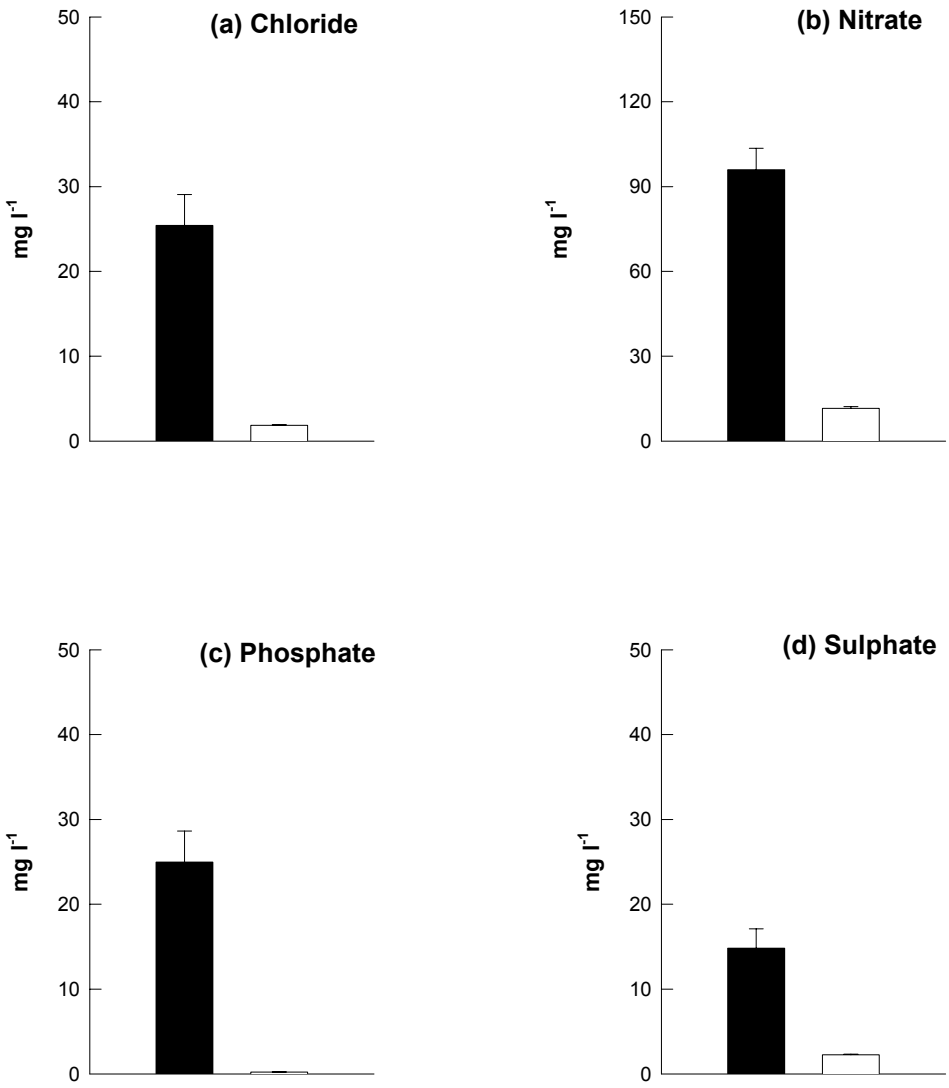


Figure 8. comparisons of nutrient availability (mg l^{-1} in aqueous extract) between May and January abandoned nest sites : (a) Chloride, (b) Nitrate, (c) Phosphate (d) Sulphate. filled bars, May, open bars, January. Error bars represent one standard error (n = 25)

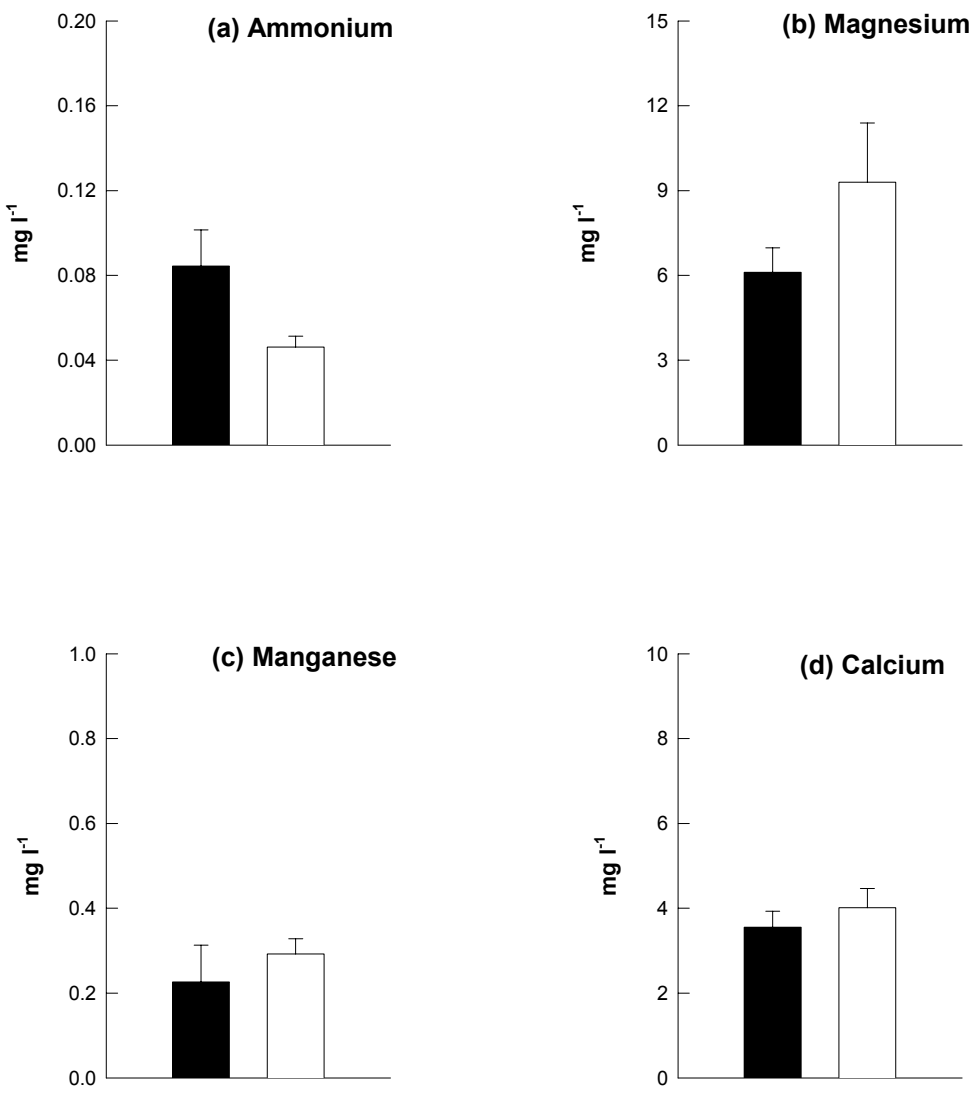


Figure 9. comparisons of nutrient availability (mg l^{-1} in aqueous extract) between May and January abandoned nest sites : (a) Ammonium, (b) Magnesium, (c) Manganese (d) Calcium. filled bars,May, open bars, January. Error bars represent one standard error (n = 25)

3.3.0 Species cover

The data was summarised as bar charts and displayed in figures 10, and 11. Significant differences between nest and non nest sites were analysed using an Independent t-test, the results from which are displayed in tables 8, and 9.

3.3.1 May 1999 samples

The plant species cover between the May sites can be seen in figure 10. The results indicate there were no significant ($P < 0.05$) difference in species cover between abandoned nest and non nest sites as shown in table 8.

Species	t	df	Nest (μ)	Nest (s.e)	Non Nest (μ)	Non Nest (s.e)	Sig. (P)
S. uniflora	-1.186	48	16.560	$\pm$ 4.281	23.960	$\pm$ 4.540	0.24
A. elatius	0.486	48	38.480	$\pm$ 4.925	35.120	$\pm$ 4.853	0.63
Cladonia	0.173	48	1.080	$\pm$ 0.326	0.960	$\pm$ 0.612	0.86
H. lutescens	0.102	48	3.040	$\pm$ 0.724	2.920	$\pm$ 0.932	0.92

Table 8. Comparisons between abandoned nest and non-nests of the species percentage cover. Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; Independent t-tests)

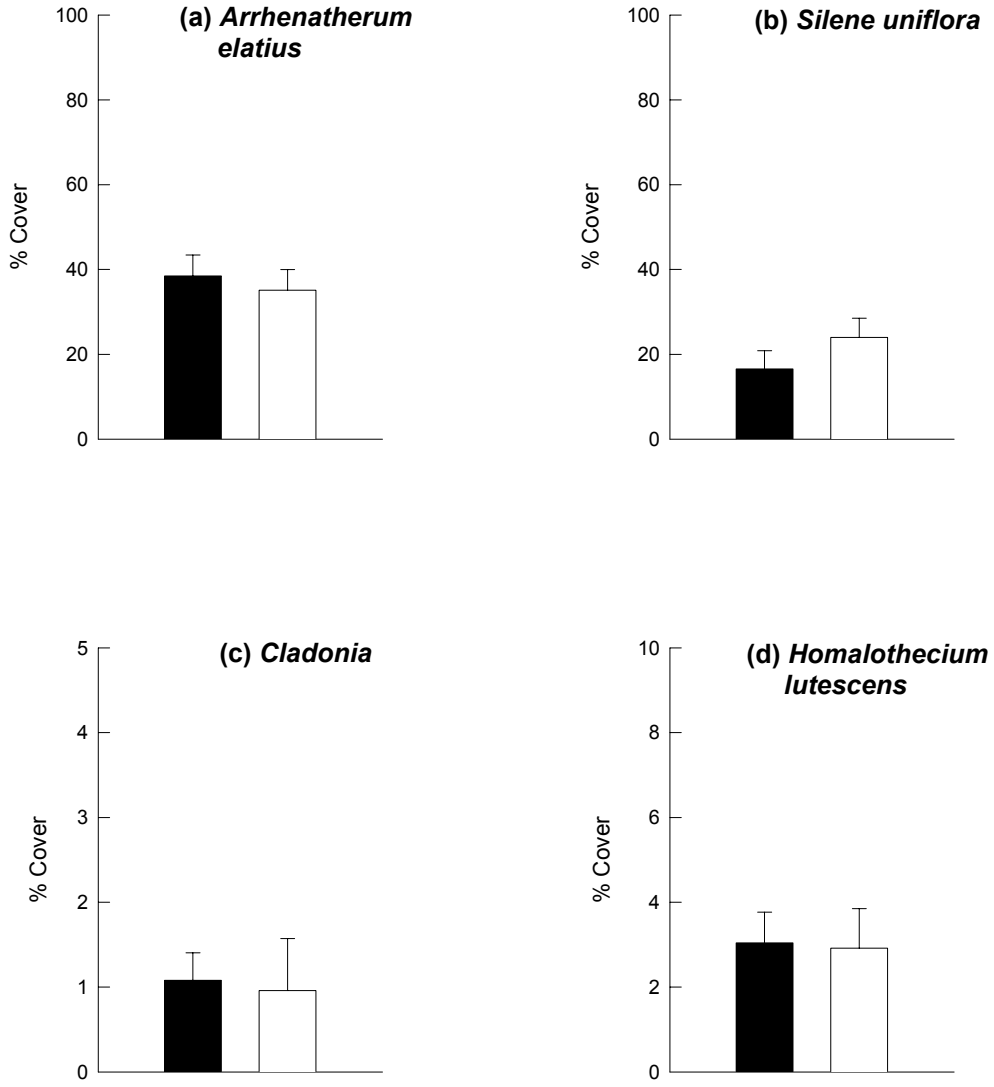


Figure 10. Comparison between Mays abandoned nest and non-nest sites of the percentage cover of (a) *Silene uniflora*, (b) *Arrhenatherum elatius*, (c) *Cladonia*, (d) *Homalothecium lutescens*. Filled bars, abandoned nest site; open bars, non-nest sites. Error bars represent one standard error (n = 25).

3.3.2 January 2000 samples

The plant species cover between the January sites can be seen in figure 11. The results indicate there were no significant ($P < 0.05$) difference in species cover between abandoned nest and non nest sites as shown in table 9.

Species	t	df	Nest (μ)	Nest (s.e)	Non Nest (μ)	Non Nest (s.e)	Sig. (P)
S. uniflora	-0.778	48	10.480	$\pm$ 2.618	13.40	$\pm$ 2.688	0.44
A. elatius	1.935	48	47.600	$\pm$ 3.417	37.00	$\pm$ 4.281	0.60
Cladonia	1.191	48	2.080	$\pm$ 0.627	1.240	$\pm$ 0.322	0.23
H. lutescens	-1.361	48	2.720	$\pm$ 0.537	4.520	$\pm$ 1.208	0.18

Table 9. Comparisons between abandoned nest and non-nests of the species percentage cover. Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; Independent t-tests)

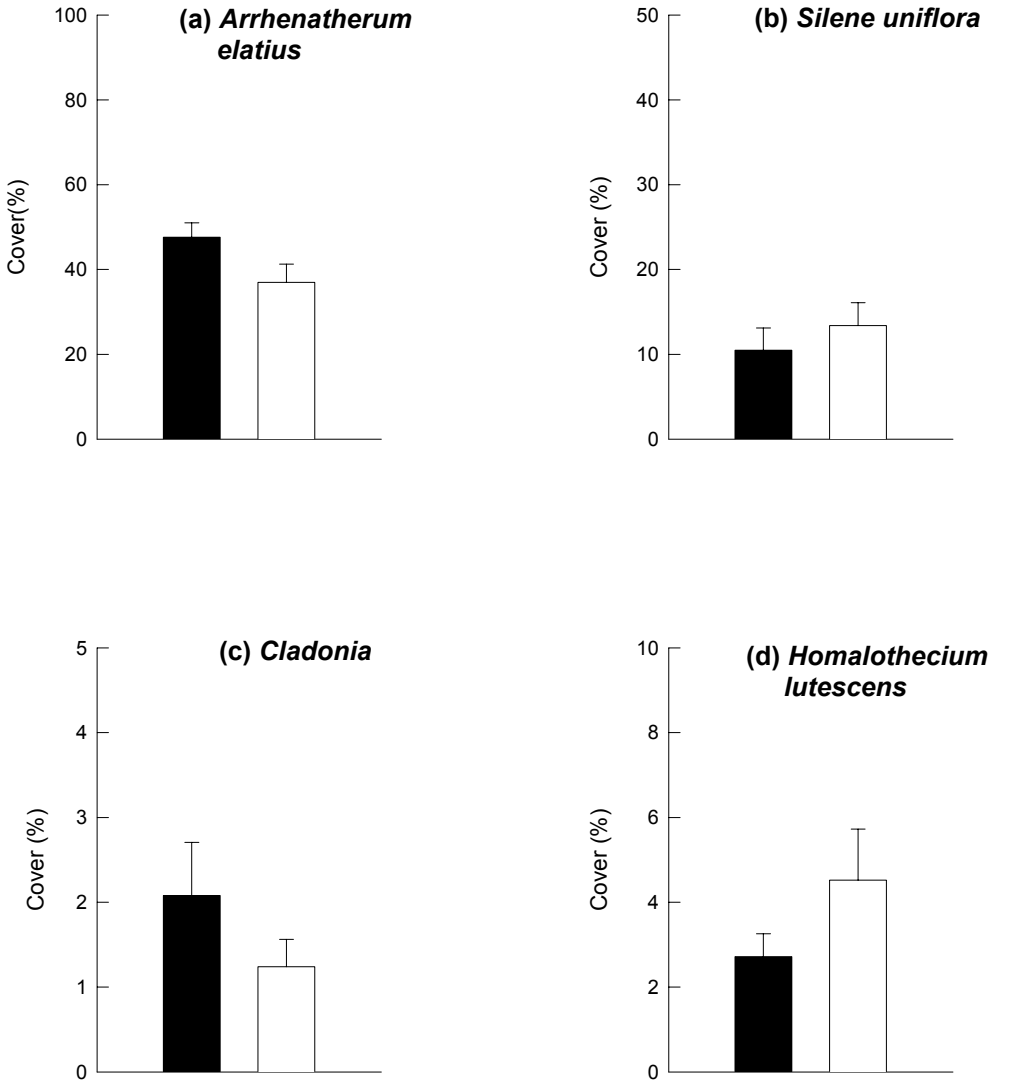


Figure 11. Comparison between Januarys abandoned nest and non-nest sites of the percentage cover of (a) *Silene uniflora*, (b) *Arrhenatherum elatius*, (c) *Cladonia*, (d) *Homalothecium lutescens*. Filled bars, abandoned nest site; open bars, non-nest sites. Error bars represent one standard error (n = 25).

3.4.0 Soil mineralization

To test whether any difference exists in the turnover of available soil nitrogen between abandoned nest and non-nest sites, the incubation data for the two available forms of nitrogen (NO_3^- & NH_4^+) were analysed. The data was summarised as bar charts and displayed in figure 12 (a, b) for nitrate, and figure 13 (a, b) for ammonium.

3.4.1 Soil nitrate

No significant difference ($P < 0.05$) in the concentration of soil nitrate existed within the two groups incubated in the laboratory, as shown in figure 12.a for nest and 12b for non-nest sites. Both groups showed a significant difference ($P > 0.01$) in nitrate concentration between the laboratory samples and those incubated on site.

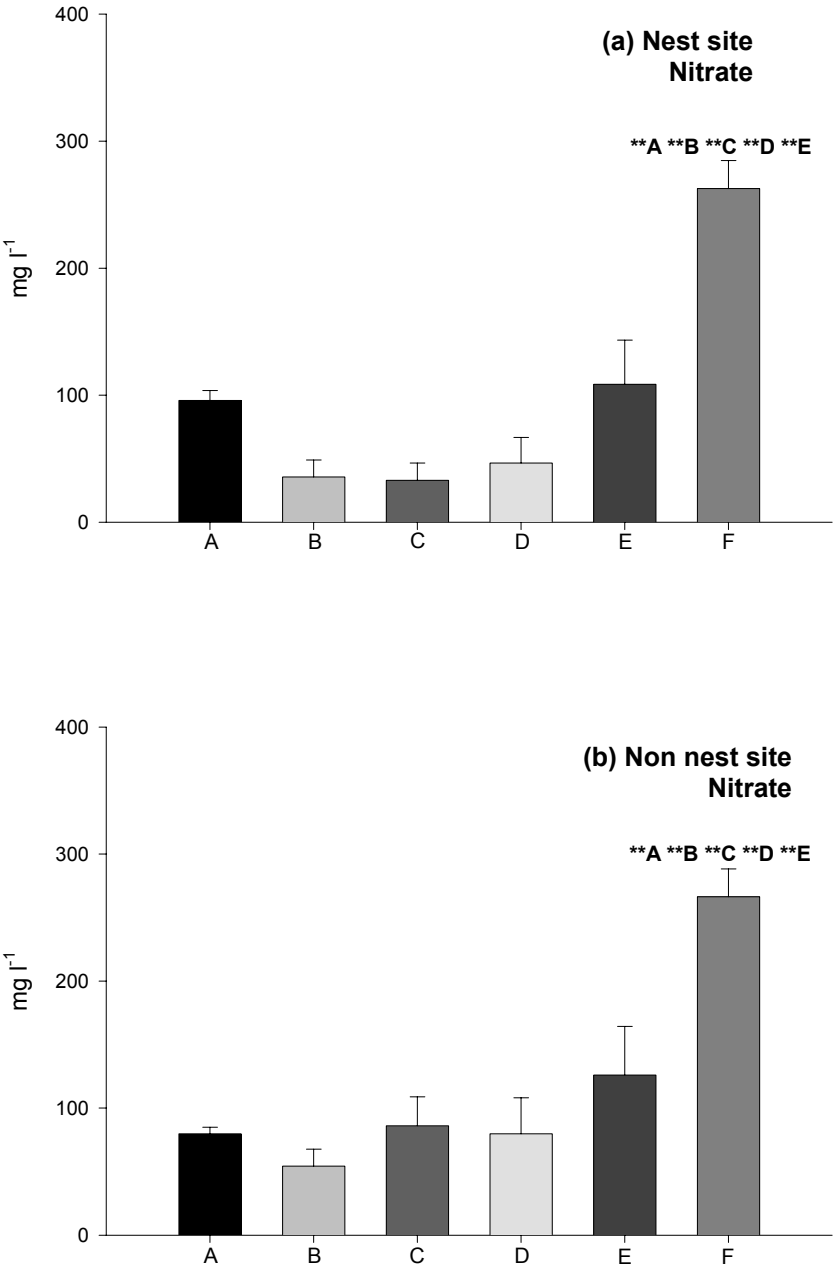
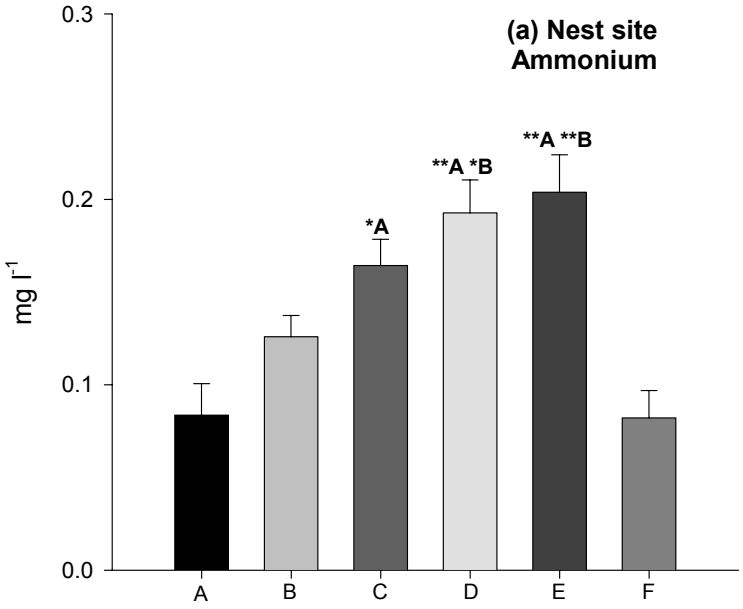


Figure 12. comparisons of nitrate levels (mg l^{-1} in aqueous solution) over a 4 week incubation period at 20^{o}C of (a) abandoned nest, (b) non-nest. Bars represent: A, initial sample, B, 1 week, C, 2 week D, 3 week, E, 4 week, F, 4 week on site. Error bars represent one standard error (n = 25). Significant differences between incubation periods are indicated by asterisks (* P < 0.05, ** P < 0.01; ANOVA, Turkey post hoc tests).

3.4.2 Soil ammonium

Both groups showed a significant ($P < 0.05$) increase in ammonium concentration over time as shown in figure 13.a, b. Increases within nest site ammonium concentration became significant ($P < 0.05$) in week two of incubation compared to non-nest site samples which did not show any significant ($P < 0.05$) increase in concentration until week three. Ammonium concentrations continued to increase significantly in both sample groups, although the increases in the nest site samples was of greater significance ($P < 0.01$) for weeks three and four. Samples buried on site at Orford Ness showed no significant increase ($P < 0.05$) in ammonium concentrations for nest and non-nest sites as shown in figure 13.a,b.



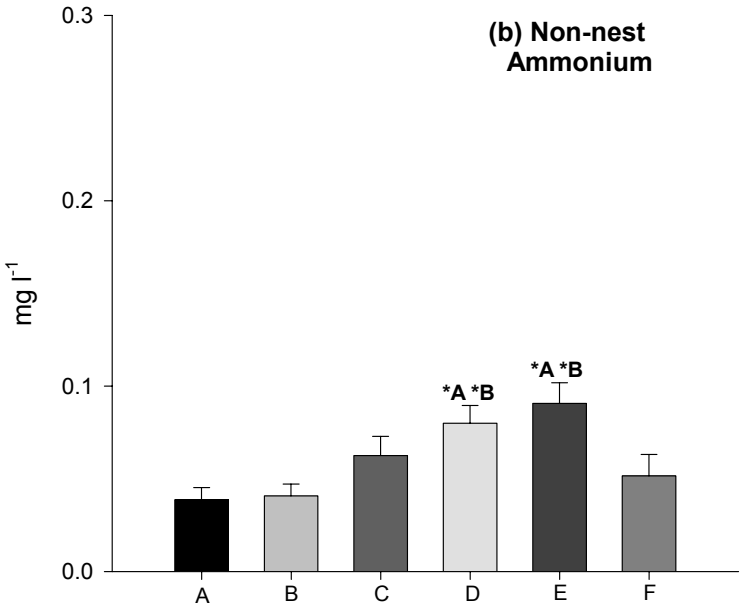


Figure 13. comparisons of ammonium levels (mg l^{-1} in aqueous solution) over a 4 week incubation period at 20^{O}C of (a) abandoned nest, (b) non-nest. Bars represent: A, initial sample, B, 1 week, C, 2 week D, 3 week, E, 4 week, F, 4 week on site. Error bars represent one standard error (n = 25). Significant differences between sites are indicated by asterisks (* P < 0.05, ** P < 0.01; ANOVA, Turkey post hoc tests).

3.5.0 Plant nutrient response

3.5.1 *Arrhenatherum elatius*

The response of *A. elatius* root biomass to the nutrient treatments can be seen in Figure 14 (a). There was no significant difference ($P > 0.05$) in shoot biomass between the treatments.

The response of *A. elatius* shoot biomass to the nutrient treatments can be seen in Figure 14 (b). A Significant increase in shoot biomass could be seen with the + N (C) treatment when compared with the control (A) ($P< 0.05$) and + P (B) ($P < 0.01$) treatments. Shoot biomass in the combined + N and + P (D) treatment showed a significant difference when compared with control, + P and + N ($P < 0.01$) treatments.

The total biomass of *A. elatius* can be seen in Figure 14 (c). The only significant increase in root biomass was for the + N (C) treatment ($P < 0.05$) when compared with + P (B). There was no significant difference ($P >0.05$) for any of the treatments when compared with the control (A).

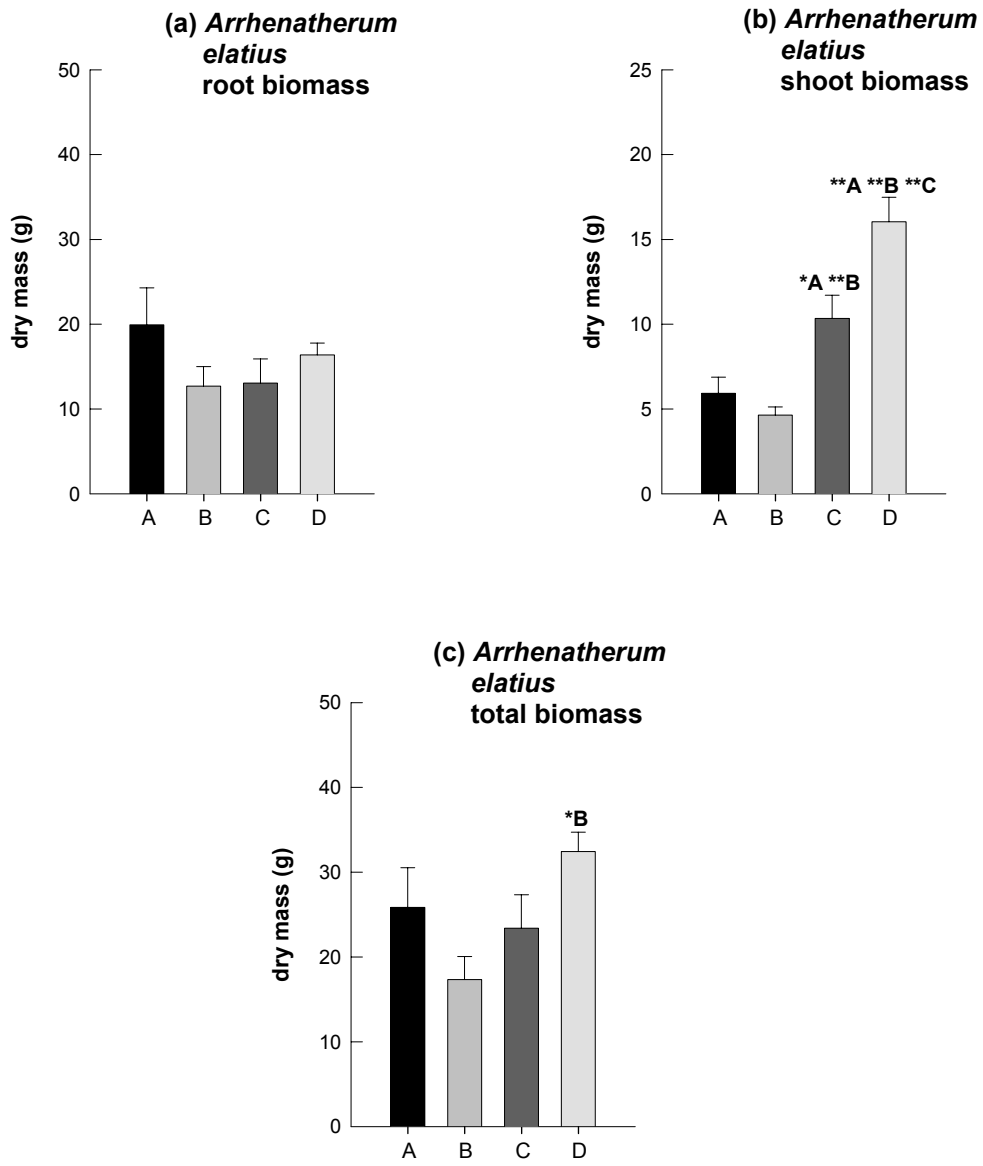


Figure 14. Response of *Arrhenatherum elatius* to nutrient treatments: (a) root biomass, (b) shoot biomass, (c) total biomass. Bars represent: (A), control, B,(+ P), (C), + N, (D), + P & + N. Error bars represent one standard error (n = 9). Significant differences between treatments are indicated by asterisks (* P < 0.05, ** P < 0.01; ANOVA, Tukey post hoc tests).

3.5.2 *Silene uniflora*

The response of *S. uniflora* root biomass to the nutrient treatments can be seen in Figure 15 (a). The only significant increase in root biomass was for the + N (C) treatment ($P < 0.05$) when compared with + P (B). There was no significant difference ($P > 0.05$) for any of the treatments when compared with the control (A).

The response of *S. uniflora* shoot biomass to the nutrient treatments can be seen in Figure 15 (b). A Significant increase in shoot biomass could be seen with the + N (C) treatment when compared with the control (A) and + P (B) treatments ($P < 0.01$). Shoot biomass in the combined + N and + P (D) treatment showed a significant difference when compared with control, + P ($P < 0.01$) and + N ($P < 0.05$) treatments.

The total biomass of *S. uniflora* can be seen in Figure 15 (c). A Significant increase in total biomass could be seen with the + N (C) treatment when compared with the control (A) ($P < 0.01$) and + P (B) ($P < 0.05$) treatments.). Total biomass in the combined + N and + P (D) treatment also showed a significant increase when compared with the control and + P ($P < 0.01$) treatments.

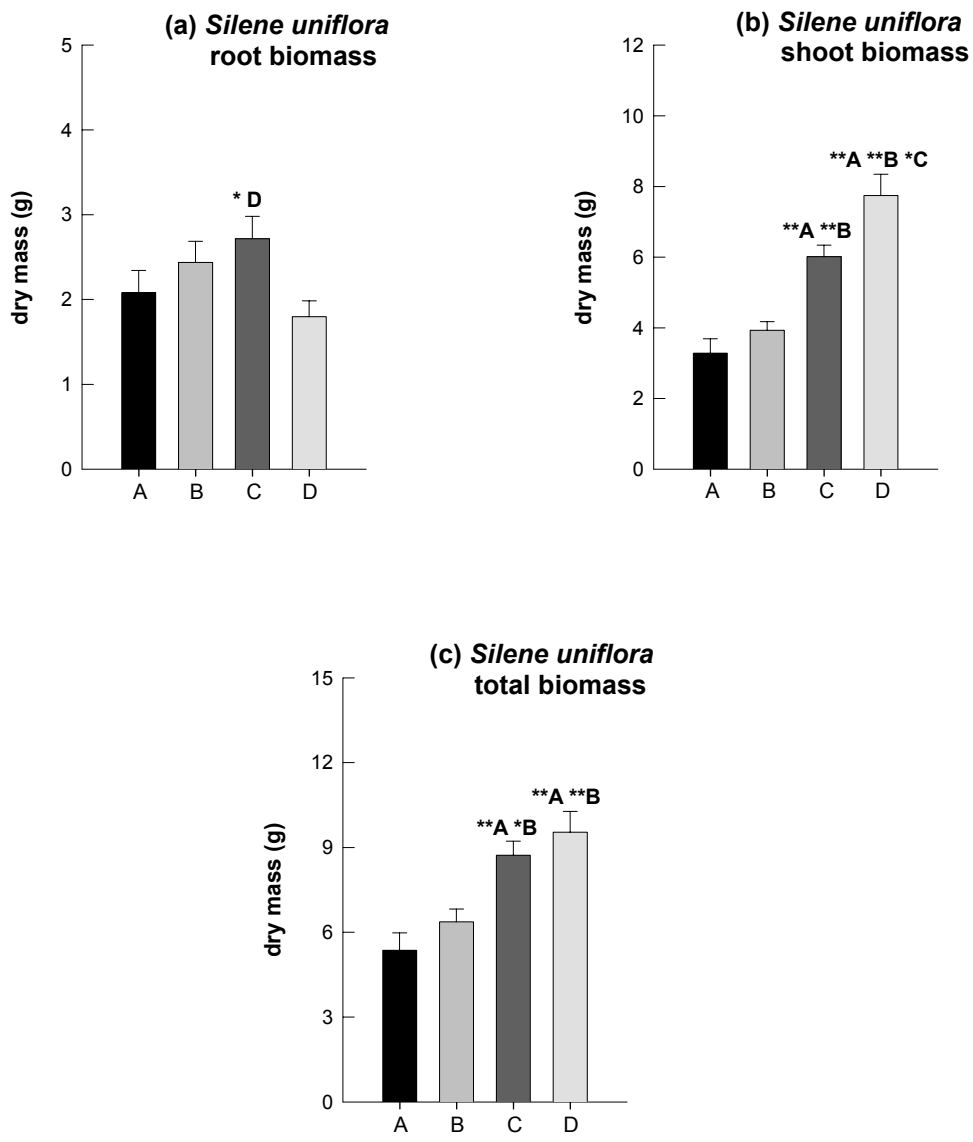


Figure 15. Response of *Silene uniflora* to nutrient treatments: (a) root biomass, (b) shoot biomass, (c) total biomass. Bars represent: (A), control, (B), + P, (C), +N, (D), + P & + N. Error bars represent one standard error (n = 9). Significant differences between treatments are indicated by asterisks (* $P < 0.05$, ** $P < 0.01$; ANOVA, Tukey post hoc tests).

3.5.3 *Arrhenatherum elatius* grown with *S. uniflora*

The response of *A. elatius* root biomass when grown with *S. uniflora* to the nutrient treatments can be seen in Figure 16 (a). There was no significant increase ($P > 0.05$) in shoot biomass between the treatments.

The response of *A. elatius* shoot biomass when grown with *S. uniflora* to the nutrient treatments can be seen in Figure 16 (b). A Significant increase in shoot biomass could be seen with the combined + N and + P (D) treatment when compared with control, + P and + N ($P < 0.01$) treatments.

The total biomass of *A. elatius* when grown with *S. uniflora* can be seen in Figure 16 (c). The only significant increase in total biomass was for the combined + N and + P (D) treatment ($P < 0.05$) when compared with + N (C). There was no significant difference ($P > 0.05$) for any of the treatments when compared with the control (A).

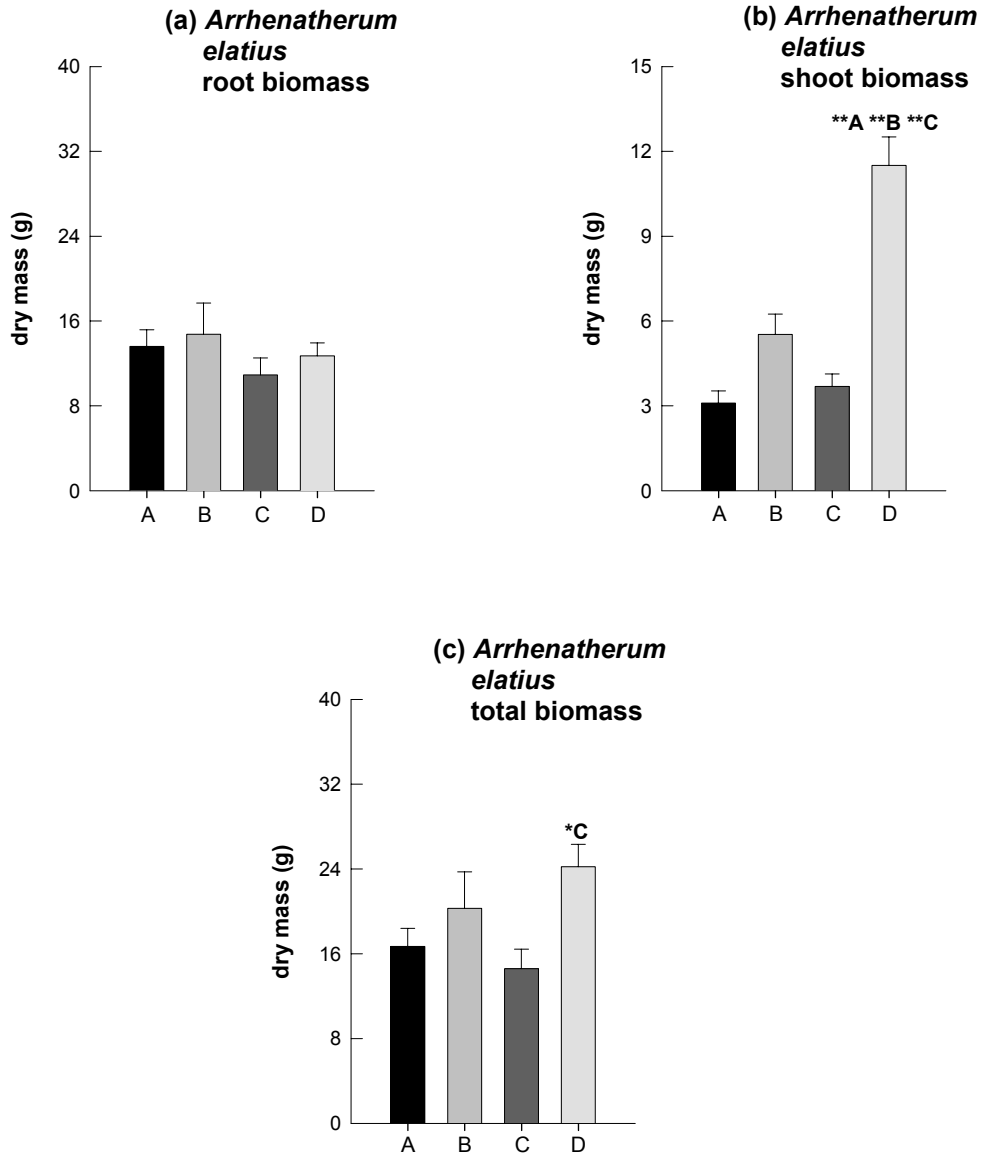


Figure 16. Response of *Arrhenatherum elatius* grown with *Silene uniflora* to nutrient treatments: (a) root biomass, (b) shoot biomass, (c) total biomass. Bars represent: (A), control, (B), + P, (C), + N, (D), + P & + N. Error bars represent one standard error (n = 9). Significant differences between treatments are indicated by asterisks (* P < 0.05, ** P < 0.01; ANOVA, Tukey post hoc tests).

3.5.4 *Silene uniflora* grown with *A. elatius*

The response of *S. uniflora* root biomass when grown with *A. elatius* to nutrient treatments can be seen in Figure 17 (a). The only significant difference in root biomass was for the + N (C) treatment ($P < 0.05$) when compared with + P (B). There was no significant increase ($P > 0.05$) for any of the treatments when compared with the control (A).

The response of *S. uniflora* shoot biomass when grown with *A. elatius* to the nutrient treatments can be seen in Figure 17 (b). A Significant increase in shoot biomass could be seen with the + N (C) treatment when compared with the control (A) and + P (B) treatments ($P < 0.01$). Shoot biomass in the combined + N and + P (D) treatment also showed a significant increase when compared with control, + P and + N ($P < 0.01$) treatments.

The total biomass of *S. uniflora* when grown with *A. elatius* can be seen in Figure 17 (c). A Significant increase in shoot biomass could be seen with the + N (C) treatment when compared with the control (A) and + P (B) treatments ($P < 0.01$). Shoot biomass in the combined + N and + P (D) treatment also showed a significant increase when compared with control, and + P ($P < 0.01$) treatments.

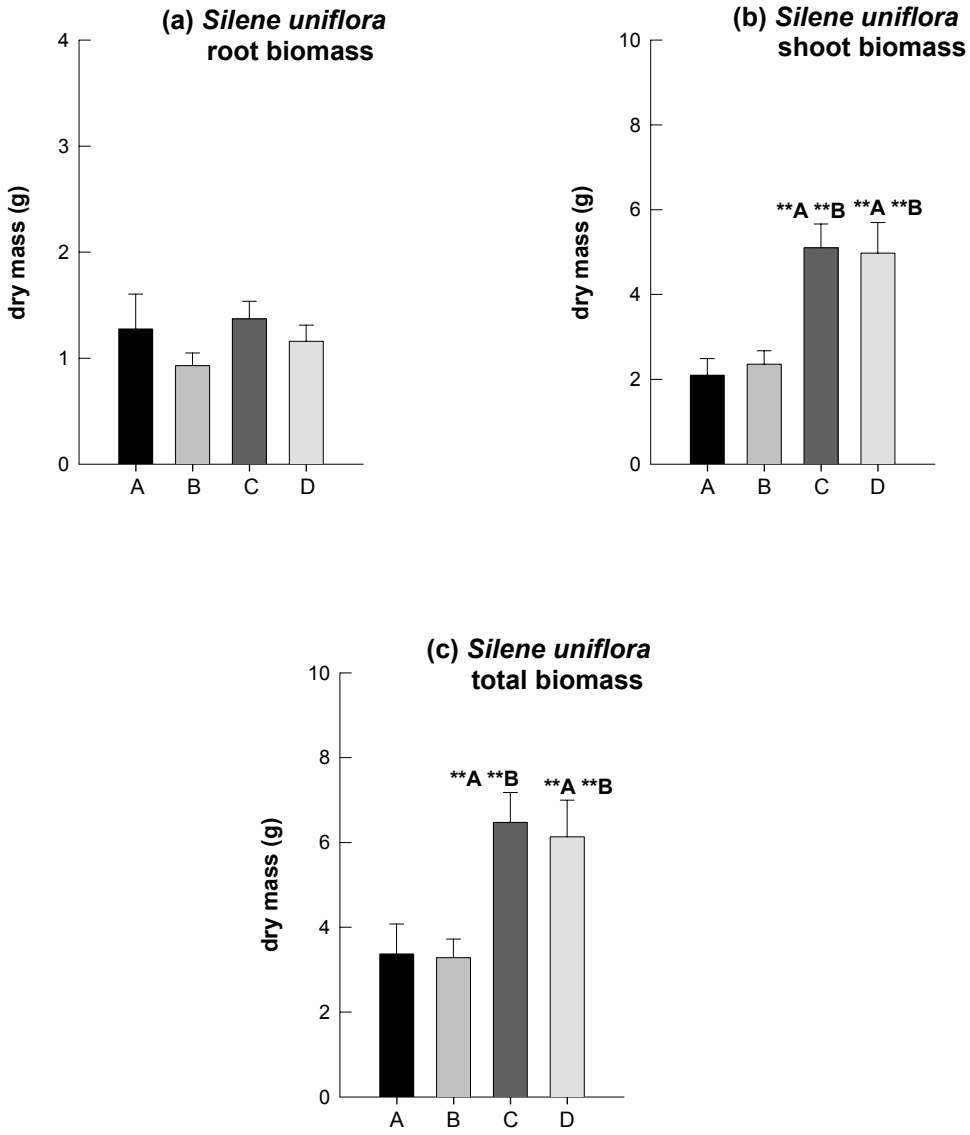


Figure 17. Response of *Silene uniflora* grown with *Arrhenatherum elatius* to nutrient treatments: (a) root biomass, (b) shoot biomass, (c) total biomass. Bars represent: (A), control, (B), + P, (C), + N, (D), + P & + N. Error bars represent one standard error (n = 9). Significant differences between treatments are indicated by asterisks (* P < 0.05, ** P < 0.01; ANOVA, Tukey post hoc tests).

4.0 Discussion

Shingle particle distribution has been shown to be one of the most dominant factors in shingle ridge vegetation establishment, with higher percentages of fine fraction resulting in higher establishment and survival rates for shingle plants (Fuller 1987). The results from the study indicate that nest and non-nest sites have a probability of plant establishment and in this respect a very similar substrate composition.

Several studies have shown gulls can increase the soil nutrient concentrations of available nitrogen, phosphorus, calcium, and magnesium surrounding their nesting colonies (Gillham, 1956; Sobey & Kenworth, 1979; Evans & Davy 2000). This increase has been related to the deposition of food scraps and chick faeces. Adult faeces is not thought to be a major contribution as adult gulls have been shown to use communal latrines on the shingle away from the nesting area (Sobey & Kenworth, 1979). The general consensus has been that these increases in nutrient concentrations have low residence times in the shingle because of over-winter leaching of the substrate (Sobey & Kenworth, 1979; Iason et al 1986). This leaching effect would be expected to be more prevalent in shingle habitats, which have a high porosity and low water retention (Davy et al. 2000).

The comparisons of abandoned nest and non-nest sites at Orford Ness in this study indicate that the gulls cause an increase in available nutrients surrounding their nest sites. May samples from abandoned nest sites showed significantly higher concentrations of phosphate ($P < 0.01$), ammonium and magnesium ($P < 0.05$) than non-nest sites (Table 5.). Although the nest sites had been abandoned for nearly one year, gull activity was widespread throughout the study site with many of the previously abandoned nests being rebuilt during sampling.
The same comparison for the following indicated that January abandoned nest sites had significantly higher concentrations for ammonium and magnesium ($P < 0.05$), with phosphate having a significantly higher concentration at non-nest sites ($P < 0.05$) (Table.6).

Comparisons between abandoned nest sites in May and January indicated a significant over-winter reduction in available nutrient concentrations for nitrate ($P < 0.01$), phosphate and ammonium ($P < 0.05$) (Table.7). These results suggest that over-winter leaching plays a major role in the reduction of available nutrient concentrations surrounding abandoned nest sites.

Earlier work by Evans & Davy (2000) showed that available nutrient concentrations for nest sites abandoned for over one year were significantly higher ($P < 0.01$) than adjacent non-nest sites. When samples were taken directly from the nest itself. Thus nutrient concentrations may remain significantly higher for abandoned nest sites in only the very restricted area of the nest. This may be due to an increase in water retention as a result of organic matter derived from nesting material.

Differences in the turnover of available nitrogen are more influential on plant nutrition composition and density of vegetation (Davy & Taylor 1973). Nitrate release on incubation did not differ significantly ($P > 0.05$) between abandoned nest and non-nest sites (Figure 12). Ammonium release was significantly ($P < 0.05$) greater for nest sites (Figure 13 a) although the concentrations involved are so much lower than respective concentrations of NO_3 that they would be insignificant in terms of plant nutrition.

One of the major concerns with nutrient enrichment on shingle is the effect it may have on shingle plant communities (Tidswell, 1993; Harding & Crewe 1994). Shingle plants have adapted to endure extreme environmental conditions such as low nutrient regimes, large temperature fluctuations and drought, and hence they tend to show slow grow and be relatively uncompetetive (Scott, 1963; Chapman, 1976; Fuller 1987). One of the major concerns at Orford Ness was the effect *Arrhenatherum elatius* might have on the shingle ridge vegetation, as it has been shown to exclude other grass species in fertilized plots (Berendse et al 1992). A major objective of this project was to discover whether *A. elatius* could out-compete *Silene uniflora* around abandoned nest sites were available nutrient concentrations where significantly higher.

The survey of species cover found no evidence of a difference in cover for *A. elatius* or *S uniflora* between abandoned nest and non-nest sites (Tables 8, 9). Similarly, cover for *Cladonia* and the moss *Homalothecium lutescens* showed no significant difference between sites. This was contrary to expectations as both species are used extensively by the gulls as a nest liner.

Could increases in nutrient availability around abandoned nest sites alter biomass in the two dominant ridge plant species *Silene uniflora* and *Arrhenatherum elatius*? Glasshouse experiments indicated that *A. elatius* responded well to nitrogen, with significant increase in shoot biomass, although root and total biomass showed no significant increase. Similar responses were seen with the addition of nitrogen and phosphorus with significant increases in shoot biomass and total biomass. (Figure 14). However *S uniflora* also responded well to nitrogen, with significant increases in root biomass, shoot biomass, and total biomass ($P < 0.01$). Similar responses were seen with the addition of nitrogen and phosphorus with significant increases in shoot biomass, and total biomass (Figure 15).

Do competitive interactions restrict either species when grown together?
A. elatius when grown in the presence of *S. unflora* only responded to the combined nitrogen and phosphorus treatment with a significant increase in shoot biomass There was no significant increase in root biomass and total biomass only showed a significant increase when compared to the phosphorus treatment (Figure 16).
S. unflora responded well to nitrogen, with significant increases in shoot biomass and total biomass even when grown in the presence of *A. elatius* . Similar responses were seen with the addition of nitrogen and phosphorus with significant increases in shoot biomass, and total biomass, although there was no significant increase in root biomass (Figure 17).

These results suggest that *Silene uniflora* is not at a competitive disadvantage in the presence of increased nutrient concentrations, and can respond well to any increase in available nitrogen, or a combination of available nitrogen and phosphate, that may result from nesting gulls.

The results from this study indicate that *Silene uniflora* in particular and potentially other shingle species in general should not be restricted by the presence *Arrhenatherum elatius* or nesting gulls, and any increase in nutrients would stimulate its growth. This brings into question whether nutrient enrichment of the shingle alone could cause changes in plant community structure.
It has been suggested that gulls may cause vegetation disturbance through territorial disputes such as boundary clashes (Sobey & Kenworth 1979). Evans and Davy (2000) showed that vegetation disturbance by gulls, as a result of boundary clashes was minimal on the shingle ridges at Orford Ness

4.1.0 Conclusion

- **Nesting gull's do cause eutrophication of the shingle habitat.**
- **Nutrient concentrations are significantly reduced over winter.**
- **Vegetation appears uniform between abandoned nest & non-nest sites.**
- **Increases in available nutrients will be utilised by the shingle plants.**
- ***Arrhenatherum elatius* is not competitively dominant on site.**
- **Vegetation disturbance by the gulls on the shingle is minimal.**

It is the conclusion of this study that nesting gulls do not cause a change in the vegetation at Orford Ness at their present density, and so it appears that the gulls and plants can coexist without extensive alteration to the shingle ecosystem.

Acknowledgements

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I thank the National Trust, Grant Lohoar, and Dave Cormack for access and help on site. I also thank Dr Anthony Davy & Mrs Eunice Evans for support & supervision.

Literature cited

Berendse, F, Elberse, W,T, Geerts, R, H, M, E. 1992. Competiton & Nitrogen loss from plants in grassland ecosystems. Ecology. Vol. 73(1).pp 46-53

Bullock. J. 1996. Plants. In Ecological census techniques: A handbook, ed. by Sutherland. W. J, pp. 111-138. Cambridge University press. Cambridge.

Carr. A. P. 1972. Aspects of spit development & decay: The estuary of the river Ore, Suffolk. Field Studies. Vol. 3. pp 633-653.

Chapman. V. J. 1976. Coastal Vegetation. Pergamon Press. London. pp 235-251.

Cormack. National Trust warden. 1999. Personal communication on gull population numbers National Trust Site. Orford Ness. Suffolk.

Davy, A.J. , Willis, A.J. & Beerling, D.J. (2000) The plant environment: aspects of the ecophysiology of shingle species. Ecology & Geomorphology of Coastal Shingle (eds J.R. Packham, R.E. Randall, R.S.K. Barnes & A. Neal), pp. 191-201, Westbury Academic & Scientific Publishing, Yorkshire. (in press)

Evans, P.C. & Davy, A.J. (2000) Effects of nesting gulls on shingle vegetation at Orford Ness, Suffolk, UK. Ecology & Geomorphology of Coastal Shingle (eds J.R. Packham, R.E. Randall, R.S.K. Barnes & A. Neal), pp. 297-302, Westbury Academic & Scientific Publishing, Yorkshire. (in press)

Fuller. R. M. 1989. Orfordness & Dungeness: A comparative study. Botanical Journal of the Linnean Society. Vol. 101. pp 91-101.

Fuller. R. M. & Randall. R. E. 1988. The Orford shingles Suffolk, UK- Classic conflicts in Coastline management. Biological Conservation. Vol. 46. pp 95-114.

Fuller. R. M. 1987. Vegetation establishment on shingle beaches. Journal of Ecology. Vol. 75. pp 1077-1089.

Gillham. M. E. 1956. The ecology of the Pembrokeshire Islands. V. Manuring by the colonial sea birds & mammals with a note on seed distribution by gulls. Journal of Ecology. Vol. 44. pp 428-454.

Green. C. P. & Mc Gregor. D. F. M. 1989. Orford Ness a geomorphological Assessment. NCC. CSD Report. No. 847.

Harding. M. & Crewe. M. 1994. Botanical survey. In Ecological Survey of Orford Ness 1993/1994, ed. by Beardall. C. Suffolk Wildlife Trust.

Kershaw. K. A. & Alvin K. L. 1966. The observers book of Lichens. Frederick Warne & CO Ltd. London.

Scott. G. A. M. 1963. The Ecology of shingle beach plants. Journal of Ecology. Vol. 51 (3). pp 517-527.

Sobey. D. G. & Kenworthy. J. B. 1979. The relationship between Herring gulls & the

vegetation of their breeding colonies. Journal of Ecology. Vol. 67. pp 469-496.

Tidswell. R. 1993. Baseline study for monitoring the effects of a gull colony on the shingle ridge vegetation at Orford Ness NNR Suffolk. English Nature. East region Science team. SSSI monitoring report. Series No. 3. 581.526(426.4)

APPENDIX 1 Shingle conference paper.

APPENDIX 2 Detailed Methods.

1. Anion analysis using Ion-Chromatography (DIONEX-DX-100)

date: 12-03-1998
author: Hans Schutten

Introduction
Analysing anions in sediment pore water can be done in various ways. Up to now the majority of the anions
were analysed using classical wet-chemistry methods. These methods are very laborious. During 1994, the
School for Biological Sciences of the UEA purchased an Ion-Chromatograph (IC) from DIONEX . Because
only anions are analysed on this machine, a fairly simple setup is chosen; a DX-100 with a conductivity
detector, autosampler and chemical workstation.

I.a making standards
To run the IC properly and quantitatively a series of standards with different concentrations have to be made. This range has to be wider than the concentrations found, or likely to be found in the samples for all
to be analysed anions.
Due to the capacity of the column, the range the equipment can handle is 0 to 100 mg/1. So the chosen
standards are:

table 1: Standard concentration range (mg / 1) for IC on DX- 100

standard	**Chloride Cl**	**Nitrite NO2**	**Nitrate NO3**	**Phosphate PO4**	**Sulphate SO4**
number	M = 35.453	M = 46.006	M = 62.005	M = 94.971	M =96.058
AutocalIR	10.0	0.50	0.50	0.50	1.00
Autocal2R	15.0	0.75	1.00	2.50	25.0
Autocal3R	20.0	1.00	5.00	10.00	50.0
80% standard	15.0	0.75	4.00	7.00	25.0

The standards are prepared out of standard solutions (1000 ppm) for Chloride, Nitrate, Phosphate and Sulphate, and out of salts (analytical quality) for Nitrite.
Keep all standard solutions in the fridge (cold and dark), be careful of the expire dates.
Make a new / clean set of standards every month

Recipe:
1. - Use thoroughly clean glassware (soak overnight in Milli-Q (Grade 1) water before use) and ultra pure Milli-Q water (Grade 1, conductivity less than 17.8 μOhm).
2. - Make up a set of 1000 ppm = 1000 mg/1 of al to be used anions:
- Use the 1000 ppm Standard solutions of Chloride, Nitrate, Phosphate, and Nitrite
- Weigh 100 mg Nitrite as $NaNO_2$ (M = 69.00) = 0,1500 g on a clean weighboat, and wash in a 100 ml volumetric flask with de-oxidised Milli-Q water, let dissolve, and fill to 100 ml. This is the 1000 ppm Nitrite standard solution. (must be de-oxidised after use, must be made new every 2 weeks).
3. - use clean volumetric 100 ml flask, and mark them as standard 1, 2, and 3 and 80% standard.
- fill the flasks with 50 ml de-oxidised Milli-Q water
- put in required volumes (ml) of each anion as described in table below

standard	**Chloride**	**Nitrite**	**Nitrate**	**Phosphate**	**Sulphate**

number	**Cl**	**NO2**	**NO3**	**PO4**	**SO_4**
	M = 35.453	M = 46.006	M = 62.005	M = 94.971	M = 96.058
standard used 1000 ppm		1000 ppm	1000 ppm	1000 ppm	1000 ppm
AutocalIR	I ml	50 µl	50 µl	50 µ1	100 µl
Autocal2R	1.5 ml	75 µ1	100 µ1	250 µ1	2.5 ml
Autocal3R	2.0 ml	100µl	500 µ1	1 ml	5 ml
80% standard	1.5 ml	75 µ1	400 µ1	700 µ1	4 ml

- fill with de-oxidised Milli-Q water until 100 ml, homogenise, and de-oxidise (using clean pipettes for every standard)

I.b processing samples

The DX-100 needs the sample to be filtered down to < 0.2 micrometer. Since the Rhizons filter down to only 2 microns, the samples have to be filtered before use on the IC.

The DX- 100 with the 5 ml vials needs 5 ml of sample.

Filling sample vial and auto-sampler:
1. Defrost sample overnight in the Ridge, if necessary ,and homogenise.
2. Filter -diluted- sample with a 5 ml syringe over a IC-grade filter (0.2 micron) in a clean vial.
3. Put IC-cap on, and push down to level with the IC-tool
4. Put filled vial in the correct place in the autosampler.

Important: page 2- 11, 2- 12 (3- 1 -3-3 for trace analysis) of DIONEX auto-sampler manual.
Picture AS40 Dionex automated sampler page 2-11, 2-12

I.c setting up ion-chromatograph
Important:
page D-5: table d-1, AS40 sample load times (auto-sampler manual)
page D-56: relay control of AS40 using AI-450 (computer interface and DX100 IC)
page 3-5: Flow chart of whole analysis, including place of Method, Timed events and Schedule

Build a method (Chapter 6 of AI-450 manual)
A method file contains instructions that tells the computer software (AI-450) how to control your chromatograph, how to collect data, and how to process that date generate a report once an analysis is
complete. The method includes a list of the names, and retention times of components in the standards that
will be used to calibrate the IC. If you already have a method, you can open this, and adapt it.
1. start IC-computer
2. double click on Method icon
3. double click on system, choose default (DX-100)
4. double click on detector 1, choose other, choose conductivity, change range in 30 and units in uS
5. change run time, to 9 minutes
6. sample rate should be 5 Hz
7. double click on directory next to save data, and choose your own directory
8. double click on detector I under data processing,

9. **Build Timed events** (manual page 6-19 .. 6-21) This file in the method file lists the time-sequenced commands executed by the computer interface to run the analysis. This file is stored under its own name in the methods file, and must be stored before a method can be run.
10. **Build schedule**

I.d transferring results

The output can be on paper per sample (as standard report, check on beforehand in OPTIMIZE on

correct calibration and interpretation of peaks, or as a result-file in ASCII or CVE (EXCEL).

the best way to do this is using the OPTMIZE program, saving the results, and print / format them to disk using the BATCH program.

Running the Ion-Chromatograph DX-100

update: 12-03-1998, Author: Hans Schutten BIO 003

0. Check in the logbook how many samples have been analysed since the last check on the guard
column (Kprime (guard column) and Mprime (main column)). If more than 250 samples ago check guard and main column performance.

1. Check gas-level on the nitrogen cylinder (cylinder pressure >25 Bar, output 80-100 PSI)
2. Switch on DX100 (switch on top /right hand side of DX-100)
3. The autosampler should come on with switching on the DX- 100
4. Switch on computer interface (switch at back, top, right)
5. Switch on AI-450 computer / chemical workstation, including screen and printer
6. Set following settings on DX- 100 top panel
a. Hi-pressure: push hi-press button, and keep it pressed until the led before the 2000 Pa lights.
When going to far, keep pressed until it lights again.
b. Low-pressure should be on (push once)
c. Range selector of the detector, should be the same as in the method program (= 30)
7. Check if there is sufficient eluent left in the bottle (approx. 20 ml per sample with minimum of 2
cm of eluent in bottle)(Open DX-100)
8. Switch gas on (inside DX100, right)
9. Check gas pressure (should be between 5 and 7 psi, if not check cylinder values again, or adjust settings by pulling the knob, and turning it, and pushing it (locking it) again.
10. Switch pump on (let run for approximate 20 minutes before first analysis run)
11. Check flow rate of eluent (small counter, low, middle. this should be on 200, if not adjust by
releasing block, and turning knob, and block knob again)
12. Close DX-100 (the system should stay at a constant temperature during analysis)
13. Open your schedule file on the computer
14. Put calibrations (autocal 1r...) at the beginning in the schedule file, and your sample afterwards,
and a stop method at the end, and save it. Recommended is a 3 level calibration (in the range you
work with) with at every 20 samples an extra calibration using a solution with contains 80% concentration of the maximum for the most important ions. After presupposed very concentrated
samples it is recommended to put a rinse vial before the next sample vial (= leave top of filter above top vial level)
15. Load the autosampler with the same samples as designated in the schedule file (racks with
black dot right front (track in front). Switch autosampler to run. First vial should move to load position.

16. If control light on interface is continuously burning on 'on line' switch the interface off and on again
17. Open run program
18. Load schedule in the run program
19. Put the control button on the DX-100 on relay (you won't here the pump stop)
20. Choose the run command in the run menu, and click on **start, and the machine should do the rest itself.**
20. Calculate the approximate time of ending (samples * 12 minutes), and come back when machine is ready.
21. Note in the note DX- 100 notebook the number and origin of the samples analysed on the machine and any problems occurred during operation).
22. During analysis the computer can be used to process the chromatograms, and results.

Analysis of Ammonium (NH4+) **in water using an Ammonium Specific Electrode.**

date: 03-07-1996 author: Hans Schutten

material needed
- air-tight sample vials
- Rhizon soils moisture samplers (Van Walt / Eijkelkamp Netherlands)
- ISAB ((Ionic Strength Adjusting Buffer) for storage and measuring under a standardised ionic strength. Recipe: 0.1M LiAc (Lithium Acetate) in RO-water. Store

in refrigerator in closed bottle.
- magnetic stirrer and 3 small magnetic followers
- 25 ml beakers
- Ammonium Selective Electrode (Sentex)
- double junction reference electrode (Sentex)
- Ammonium Chloride (salt, analytical grade quality)
- Filling solution of Ammonium ISE (small white bottle)
- Outer chamber filling solution of the reference electrode (lower hole) = 0.1M LiAc
- Inner chamber filling solution of the reference electrode (upper hole) = 3M KCl

sampling
Sample with Rhizone, avoid contamination with excessive air.

Storage
in air-tight veils, in the refrigerator for maximum of 3 days in ISAB
3 ml of sample in 12 ml of ISAB.

Analysis
- flush inner chamber of reference electrode using a syringe with thick hypodermic needle
- flush outer chamber of reference electrode
- refresh liquid in the Ammonium ISE
- connect electrodes to the mV-meter, and switch on mV-meter
- let the temperature in the samples adjust to room temperature.
- pour samples in 25 ml beaker
- place beaker + magnetic follower on stirrer
- insert both electrodes in the fluid.
- read mV on the mV-meter
- empty sample in waste beaker and pour the next sample in the beaker
- calculate Ammonium concentration using the calibration curve.

Calibration.
- make up standards (4) in 4M Lithium acetate, in the range of concentrations you expect to find (check on previous calibration curves)
- measure mV in triplicate as described above
- construct calibration curve in Excel (curve-fit) with mV on x-axis and -log concentration on y-axis.
- get curve equation from Excel, and use it to calculate sample concentrations.