# Stress tolerance of *Ranunculus acris* and *Sanguisorba officinalis*

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**Summary**

Nature development is a new feature in nature conservation. In nature development large areas are turned into core areas containing target vegetation. A target vegetation contains different plant species. Some of these species are common and other are rare. In this research the attention goes to a rare species: *Sanguisorba officinalis*, and a more common one: *Ranunculus acris*. The aim of the present experiment is to determine whether a difference in tolerance to certain stress factors can explain the fact that *Sanguisorba officinalis* is a rare species and *Ranunculus acris* a more common one.

The tolerance of these two plant species is tested by exposing them to two a-biotic stress factors: high chloride levels and sulphide. Competition for light between plants is simulated by reducing the light level. These three factors were introduced to plants growing on three soil types; clay, peat or sand to relate the experiment to a nature development project in Midden-Groningen (The Netherlands). By exposing the plans to these three factors the hypothesis is tested that the light level is the main factor determining plant establishment.

According to analysis sulphide production did not occur however, but the other two stress factors were present.

The results show that a high chloride level (10.6 mmol/l) is a stress factor that affected the biomass production and the survival of both species. Reducing the light level on the other hand did not have an effect on these parameters. This factor resulted mainly in adaptations of plants to shading; such as investments in length growth and in leaf area.

*Ranunculus acris* showed a pronounced preference for the clay soil. On the other soil types it showed a very low survival.

Summarising *Ranunculus acris* was more affected by the stress factors than *Sanguisorba officinalis*. This means that in this case the common species was less tolerant than the rare species. Therefore the hypothesis that this species is rare because it has a lower tolerance has to be rejected. The 'rareness' of *Sanguisorba* may be caused by differences in tolerance towards other (stress)factors, differences to the stress factors (in the present experiment) in other life stages or problems in dispersal.

A reduced light level did not affect the plants as negatively as the higher chloride level. In this case the light level is not the main factor determining plant establishment.

The fact that the plants showed problems when faced with high chloride and/or sulphate levels when grown on the different soil types may imply that these species will encounter problems when introduced to the project area of Midden-Groningen, where the same conditions can be found.

## 1. Introduction

Nature development is a new development in nature conservation strategy in the Netherlands. In nature development projects, core areas are created and connected by corridors. If core areas are too far apart, new ones are created. In Midden-Groningen (The Netherlands) a new core area (1850 hectares) will be turned into different habitat types such as meadows, forests and bogs (Van Diggelen *et al.* 2000). Some parts of the area will need more managing than others, and some need more conversion because at present they are agricultural lands. Plans include the development of several vegetation types, which are important because of their natural values. These vegetation types will further be referred to as 'target vegetation'. A target vegetation is desirable for the rare species it contains. More knowledge on these species is helpful for improving the methods of nature conservation and restoration. An example of a target vegetation for Midden-Groningen is the *Fritillario alopecuretum pratensis* (lowland hay meadow). This is a meadow on humid, relatively nutrient rich soil (Schaminée *et al.* 1996). It may be found on locations with a raised water level in winter, but it also tolerates superficial desiccation in summer. The soil may be clay, but can also be a mixture of peat and clay. A plant species typical of this vegetation type is *Sanguisorba officinalis* (great burnet). This species is uncommon in the Netherlands. Another plant species, which has a high frequency in the Fritillario alopecuretum pratensis, is *Ranunculus acris* (meadow buttercup).

In the present experiment *Sanguisorba officinalis* will be considered as the 'rare' species. *Ranunculus acris* is chosen because it is a common species, which is part of many other vegetation types. When a species is common it is present in many habitats and often in great numbers. Some are rare and restricted to specific habitats only. The extent in which plants occur and the 'behaviour' they show has lead to designating different plant species with a 'strategy'. One of these classifications is the one of the r- and K- selection (MacArthur & Wilson 1967). This group describes the two extreme ends of a continuum. At the 'r-side' the species can take advantage of changing conditions and increase in numbers rapidly. This means that these species are good in colonisation but they are weak competitors. Species, which have a K-strategy, are good competitors but don't perform well under situations with a high disturbance.
What is determining the difference in occurrence between *Ranunculus acris* and *Sanguisorba officinalis*? Why is the last one rare and the first very common? One aspect might lie in their tolerance. This may be the physiological tolerance of the environmental conditions, for example the water level. When a plant does not meet the required conditions it suffers from stress. The performance of the plant is determined by the tolerance towards these abiotic factors. Tolerance may, on the other hand, also imply the ability to cope with lowered light levels under dense canopies of other plants or trees. Interaction between abiotic stress on one side and light competition on the other hand determines the tolerance range of a plant.
Another trait that can affect the frequency of a species is its dispersal. A species can reproduce vegetatively or sexually. In the last case characteristics of the seeds such as the quantity and the morphology determine the success of dispersal. In this experiment however the attention goes to the first factor: tolerance. Both the biotic and abiotic tolerance are tested.

The aim of this research is to test if certain stress factors affect the establishment of certain plant species. This is done by exposing the two earlier mentioned plant species to a number of stress factors. The tolerance to stress factors varies during the stages of a plants' life. Overcoming a stress factor during germination and in the seedling stage is no guarantee for growing into the adult stage and being able to reproduce. In this experiment seedlings of *Ranunculus acris* and *Sanguisorba officinalis* are exposed to three different (but also interacting) stress factors: a reduced light level, addition of sulphate and a high chloride level (all factors are introduced to plants growing on anoxic soils). The first one is chosen because it may be the main factor determining establishing success (Kalmbacher & Martin 1983, Prach *et al.* 1996, Bos 1998, Jensen & Meyer 2001, Kotowski 2001). The last two are chosen because they are typical of (former) coastal areas like the future reserve in Midden-

Groningen. High chloride levels have been measured there and sulphide production is likely to occur in areas that have a temporal or a permanent high water level. When soils are waterlogged, the oxygen saturation decreases quickly because the diffusion of oxygen into these soils is 10.000 times slower than without waterlogging. The available oxygen will soon be depleted by the activity of aerobic micro-organisms. After that the anaerobic bacteria will take over and start breaking down organic matter. At the same time (an)organic compounds are used as electron acceptors. When the redoxpotential is still rather high (350-200 mV) $NO_3^-$ and $N_2$ are used. When these and the next acceptors in the chain are used up and the redoxpotential is lowered to values between –50 and –150 mV, sulphate is reduced to sulphide (Scheffer & Schachtschabel 1984).

As mentioned above, the first stress factor is light stress; in this case a reduced light level. This is known to have a negative effect on plant growth (Kotowski *et al.* 2001). The capability of coping with a lower light level was the reason for Grime & Jeffrey (1965) to divide plant species in two groups that have different strategies to survive. The first group shows an increased length growth in full light. This group has serious problems in surviving under a reduced light level. The other group can cope better under these conditions. By growing slowly the plants can endure long periods of shade. The result of the present research gives a better insight in the competitive response of these species (the capability of tolerating competition).
A second stress factor that is used is sulphide. Although some plants are resistant to this stress, most plants respond to it with a reduced oxygen release and a decreased nutrient uptake by the roots (Allam & Hollis 1972, Joshi *et al.* 1975). Plants exposed to sulphide stress also show a reduced root growth, fewer side roots and chlorosis (Havill *et al.* 1985). During chlorosis the plant is not able to produce enough photosynthetically active pigments. This can result in a chlorophyll deficiency, which lowers the rate of photosynthesis. The overall effect of sulphide stress is a reduced biomass production (Koch & Mendelssohn 1989).
A third stress factor is salt stress. Large amounts of $Na^+$ and $Cl^-$ affect both photosynthesis and respiration processes. Therefore this factor has negative effects on the growth rate and consequently on the biomass production (Larcher 1995). As in light stress and sulphide stress there are plant species resistant to this stress, but these may also show a negative response as a reduced growth rate of the stem and root (Ramoliya & Pandey 2002-2003). In the experiment the plants are exposed to a high chloride level.

Supplying water with high levels of sulphate and chloride is not only associated with stress but also with the process of 'internal eutrophication' (Koch & Mendelssohn 1989, Lamers *et al.* 1996, Beltman & Van de Krift 1997, Lamers *et al.* 1998, Beltman *et al.* 2000, Lamers *et al.* 2001 & Lamers *et al.* 2002). Most times this phenomenon is related to problems in nature conservation when river water is supplied to desiccating nature reserves. This supply water may not have the same composition as the water that is present in the reserve. The eutrophication is the release of adsorbed phosphate, which can be used for plant growth. Several processes are responsible for this eutrophication. The first one is bicarbonate enrichment that enhances the mineralisation rate. The second is the competition of chloride and sulphate with phosphate for adsorption places (Beltman *et al.* 2000). The third mechanism is the formation of reduced sulphur that can bind to iron. The phosphate is than released from the iron. Besides testing the tolerance of plants to sulphide and chloride, the possible internal eutrophication will also be examined.

The two plant species are in this experiment exposed to light-, sulphide-, and chloride stress aiming to answer the questions: *Do the imposed stress factors potentially limit establishment of target species and does the rare species suffer more from these factors than the common species?*
The hypothesis that the light availability is the main factor determining the successful settlement of a plant will also be tested. This may mean that a shortage of light has more

extreme results than stress caused by an increased level of sulphide and/or chloride. To reach a conclusion the impact of each stress factor will be examined separately but also in combination with the others. The results will also be related to the project area in Midden-Groningen.
Survival, changes in the biomass of the shoot and leaf area and plants lengths are used to quantify the effects of the different treatments.

## 2. Methods

2.1 Plants

Two plant species are used in this experiment. These two species are selected on the basis of the following criteria: they must both be species which may be found in a meadow-type habitat and one of them must be a rare species and the other one a more common one. They must also show comparable characteristics such as the reproductive strategy, i.e. they should both reproduce sexually. Another criterion is that they both are dicotyledonous plants because grasses or sedges could show totally different reactions to the stress factors. Two plants that answer to these demands are *Sanguisorba officinalis* and *Ranunculus acris*.

*2.1.1 Ranunculus acris*

This hemicryptophyt can be found in meadows on moist, nutrient rich soils, although it avoids the driest and wettest soils. It reaches a height of 30 to 90 centimetres and has hairy, handshaped leaves. The yellow flowers appear from April into autumn (even winter); cross-pollination leads to seed set (it has the capability of vegetative reproduction but reproduction by seed happens more often). It is a common plant in large parts of Siberia, East Asia and the upper North West of North America. It's also a common plant for Europe and can be found frequently in the Netherlands (Van der Meijden 1996, Weeda *et al.* 1985).

*2.1.2 Sanguisorba officinalis*

This species is also a hemicryptophyt that grows in meadows on wet to moist and relative nutrient rich soils and also by the waterside. This plant tolerates places that are quite wet in winter but show some superficial desiccation in summer. It's mostly found on mixed soils; combinations of sandy clay and/or peat, thereby it avoids extreme calcareous or acid soils.
It has a branched structure and grows from 30 centimetres up to 1 meter. The flowers are cylinder shaped, dark purple and appear from June to September. When pollinated it reproduces from seed. *Sanguisorba officinalis* can be found in Alaska and large parts of Eurasia, but is quite rare in the Netherlands (Van der Meijden 1996, Weeda *et al.* 1985).

The seeds of these plants were harvested in summer and stored dry and dark at 4°C. Next they were stratified under moist conditions at 4°C. They were then placed in a germination chamber in which they were exposed to alternating 12-hour periods of light at 25°C and dark at 15°C. To prevent the seedlings from growing any further before the beginning of the experiment, they were placed back at 4°C. The plants were taken out of this room the day before the start of experiment to adjust to a higher temperature.

2.2 Soils

Three soil types that occur in the project area in Midden-Groningen were chosen: clay, peat and sand.
The upper layer of the soil was cut to fit a PVC pipe (maintaining the ambient structure) with a diameter of 7.5 cm and a length of 10 cm. Most times there was no vegetation growing on the soil and in case there was, plants and roots were removed as best as possible. The cut soil cores were directly put in the pipes and stored (in an open greenhouse) outside until the beginning of the experiment. Placing them in plastic containers and subsequently piling them up partly prevented evaporation. The pipes were sealed from below with a perforated piece of plastic. These perforations made water circulation in and out the soil cores possible. Some extra soil cores were analysed to examine the moist content, pH and N/P content (table 2-1).

| Soil type | Moist content (%) | pH ($H_2O$) | pH (KCl) | Organic matter % | Total P-content % | Total N-content % |
|---|---|---|---|---|---|---|
| Clay | 22 ± 1 | 7.3 ± 0.4 | 6.4 ± 0.4 | 6.2 ± 0.4 | 0.19 ± 0.01 | 0.23 ± 0.02 |
| Peat | 74 ± 3 | 3.9 ± 0.1 | 3.7 ± 0.1 | 73.5 ± 4.3 | 0.16 ± 0.02 | 1.24 ± 0.13 |
| Sand | 11 ± 1 | 5.2 ± 0.3 | 4.4 ± 0.2 | 3.9 ± 0.3 | 0.03 ± 0.00 | 0.06 ± 0.02 |

Table 2-1. Characteristics of the soils used in the experiment (n=10).

2.3 Climate rooms

The experiment took place in two climate rooms, enabling two different light levels. This was done by covering the ceilings with a plastic screen. This screen (LEE Filters, no. 121) simulates a canopy, which reduces the light availability. The exact intensities of the light (at each wavelength) transmitted by this filter are shown in figure 2-1. The intensities (i.e. photoactive radiation) were measured on three places in each climate room with a spectroradiometer (Model SR, by Cenco® Instrumenten MIJ NV). The overall effect of this filter is a 65% reduction in light intensity. It shows a reduction in intensity especially of the wavelengths (80% light reduction in the blue region and 70% in the red region) at which the photosynthetic activity is highest (Taiz & Zeiger, 1998). This means that covering the ceiling with the filter can simulate a canopy in which the light availability for the seedling is reduced. The treatment in which the filter is used is also called 'dark', the treatment with no filter is called 'light'. Covering the ceiling did not influence the air circulation.

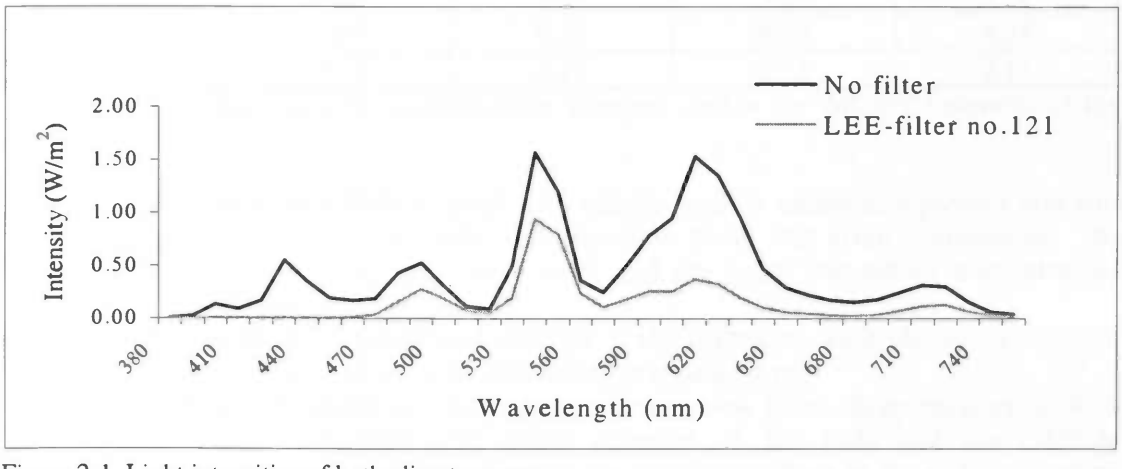


Figure 2-1. Light intensities of both climate rooms.

The climate rooms were programmed to have a 12-hour diurnal photoperiod with a temperature of 16°C at night (humidity of ± 66%) and 21°C in the daytime (humidity of ± 56%).

At the beginning of the experiment the pots were planted with seedlings of *Sanguisorba officinalis* and *Ranunculus acris* and placed on a rag-cloth in a plastic container. The rag-cloth facilitated water circulation into the soil. The inner measures of the plastic containers are 37x57x10 cm and per plant species 8 replicates were used (leading to 8 replicates per treatment). Each container was prepared with an inlet and an outflow opening, which kept the water level at ± 1.0 cm below the ground surface (top of the PVC pipes).

2.4 Treatments

Each container received a solution, which contained the salts that had to produce stress. Sulphate was added, which had to be turned into sulphide because of the high water level.

The other stress factor, chloride was also given at a high level. To prevent the cations from becoming an extra stress factor (and thereby complicating the experiment), chloride and sulphate were distributed over four different salts (with the anions sodium, potassium, calcium and magnesium). Two stock solutions (one for sulphate and one for chloride) were made which were concentrated (10x) solutions of sulphate and chloride. Each container had it's own barrel with 10 litre of solution. Each barrel contained 2 litres of water from the public water supply, one litre of the stock solution(s) and the remaining was filled up with distilled water.

Eventually there were four treatments:
1. a high level of sulphate (no extra chloride)
2. a high level of chloride (no extra sulphate)
3. a high level of sulphate and a high level of chloride
4. a control (no extra sulphate or chloride)

The exact amounts are shown in table 2-2. These treatments are called s/-, -/c, s/c and -/- respectively.

| **Ion** | **Control -/-** | **Sulphate s/-** | **Chloride -/c** | **Combination s/c** |
|---|---|---|---|---|
| $Ca^{2+}$ | 0.72 | 1.22 | 1.32 | 1.82 |
| $Mg^{2+}$ | 0.12 | 0.62 | 1.22 | 1.72 |
| $K^{+}$ | 0.04 | 1.04 | 1.74 | 2.74 |
| $Na^{+}$ | 0.46 | 1.46 | 3.16 | 4.16 |
| $Cl^{-}$ | 0.55 | 0.55 | 8.35 | 8.35 |
| $SO_4^{2-}$ | 0.11 | 2.11 | 0.11 | 2.11 |

Table 2-2. Ionic composition (in mmol/l) of the solutions used in the different treatments of the experiment.

Each container had its own 10-litre barrel with solution and by means of a pump a litre was pumped in each day. This had to make sure the stress factor was given continuously. The solution in the barrel was replaced every week and the pump and tubing were checked frequently to prevent clogging.
The concentrations of the sulphate and chloride in the containers were checked every two weeks to make sure the conditions were maintained at a stable level.
To determine to which degree the sulphide production took place, three parameters were analysed: the oxygen saturation, the redox potential of the soils and the sulphide concentration. The first two give indications on the actual conditions in the soils and can be used to decide if the experimental set-up was effective. As mentioned earlier, soils that are anoxic as a result of a high water level, may be sources of sulphide. The soils in this experiment had to be anoxic to have the same effect. The redox potential is also a parameter, which may give insight in the processes going on in the soil. Sulphide production takes places at redox potentials that are very low; the $E_h$ must have a value below zero at least (Scheffer & Schachtschabel 1984, De Mars & Wassen 1999). These parameters were measured with micro-electrodes.
To analyse the process of internal eutrophication, samples of the pore water were taken with soil samplers to examine the phosphate level and pH. These soil analyses were only possible for the peat and the sand soil, because the clay was too solid to pierce.
As a test one group of plants were grown on potting compost in the full light treatment, without extra sulphate and chloride. This was done to check the plant growth under mostly optimal conditions. These plants were not used for further analysis.

2.5 Plant analysis

The experiment lasted 65 days. After 25 days the lengths of the plants were measured. These lengths are the total lengths, being the sum of the stem and leaf of the longest offshoot of each plant. This measurement was repeated after 60 days. After 65 days the shoot of each plant was cut off. The leaves were separated from the rest of the shoot and the total leaf area of each plant was determined with a LiCor photoelectric leaf area meter.
The dry weights of stem and leaves were measured after drying at 80°C for 48h.

2.6 Statistics

The lengths and dry weights of the plants were expressed in the following parameters: dry weight (DW= total dry weight of the shoot), leaf area ratio (LAR= leaf area/total dry weight of the shoot), leaf weight ratio (LWR= leaf area/ total dry weight of the shoot), leaf area/leaf weight (LA/LW), length (length after 25 and after 60 days), mortality (number of dead plants after 25 and 65 days).
These parameters were further analysed with SPSS for Windows© (version 11.0.1). First the results of each treatment were checked for extreme values by making boxplots of the data of each parameter. These extreme values were discarded and the remaining data were then tested for normal distribution with the Shapiro-Wilk test and for homogeneity of variances with Levene's (Zar 1999). The data of most treatments did not show a normal distribution and in half of the cases the test for homogeneity of variances was not possible due to small sample sizes (i.e. in some treatments the number of dead plants was too high).
For these reasons a non-parametric analysis of variance (Kruskal-Wallis) was used for further examination of the parameters.

## 3. Results

3.1 Sulphide analysis

According to measurements the sulphate concentration in the containers was 2.2 mmol/l for the treatment with the high sulphate level and 0.2 mmol/l for the treatment with the low sulphate level.

*3.1.1 Oxygen saturation*

The graph (figure 3-1) shows quite clear the variation in the values. Though some values are very low, most of them are at least at 10% saturation or higher. That means that most soils still contain oxygen.

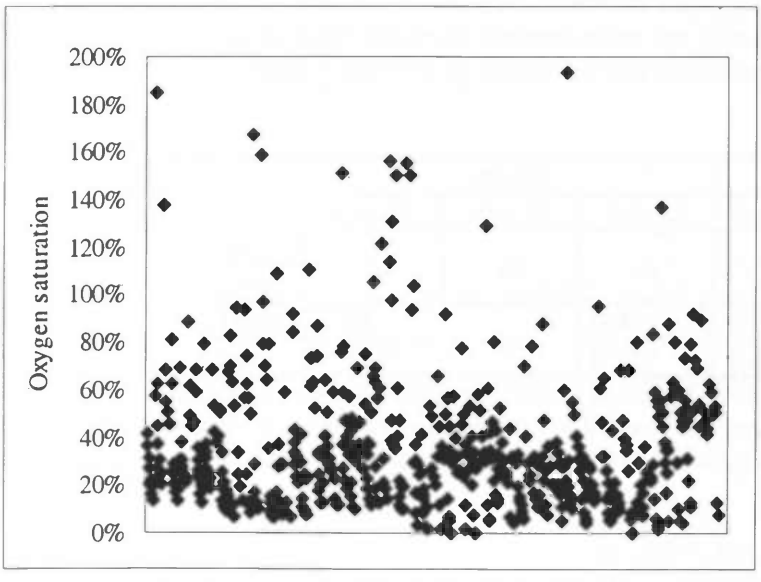


Figure 3-1. The oxygen saturation of the peat and the sand soils measured with the micro-electrodes. (sample size= 358)

*3.1.2 Redox potential*

Again there is a large scatter visible (figure 3-2). Values lower than 170 mV are not produced however.

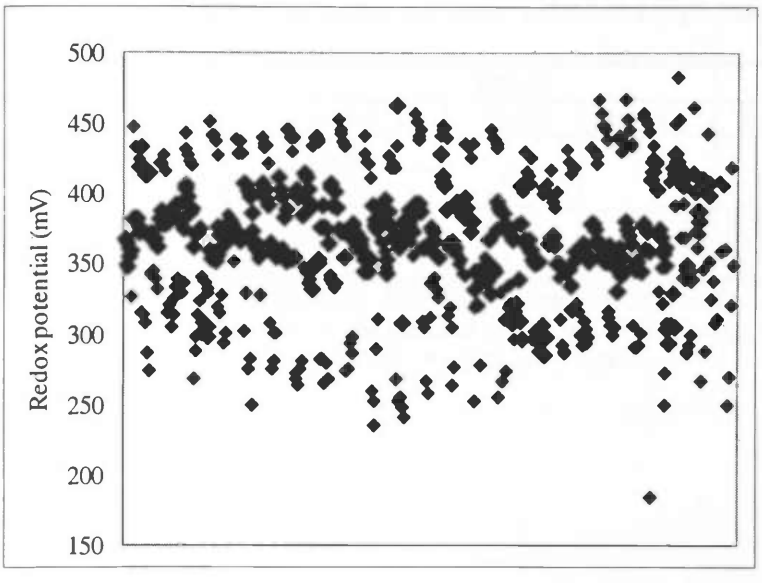


Figure 3-2. The redox potential of the peat and sand soils measured with the micro-electrodes. (sample size= 358)

*3.1.3 Sulphide level*

No detectable amounts of sulphide were measured.

3.2 Chloride analysis

Measurements of the chloride levels in the containers show a concentration of 10.6 mmol/l for the treatment with the high chloride level and 0.6 mmol/l for the treatment with the low chloride level.

3.3 Internal eutrophication

*3.3.1 Phosphate level*

In the peat soil the sulphate level does not have an effect on the phosphate level. The difference in chloride level does affect the phosphate level. When a higher chloride level is supplied, the phosphate level decreases. This is true for day 35 and day 62.

Also on the sand soil the sulphate level does not affect the phosphate level. The chloride level does have an effect. Adding chloride again results in a reduction of the phosphate level in the soil pore water.

| | | PEAT | | | SAND | | |
|---|---|---|---|---|---|---|---|
| | | day 35 | day 54 | day 62 | day 35 | day 54 | day 62 |
| Sulphate level | High | ns | ns | ns | ns | ns | ns |
| | Low | | | | | | |
| Chloride level | High | 0.04 | ns | 0.40 | 0.01 | 0.06 | 0.09 |
| | Low | 0.19** | | 0.95* | 0.27* | 0.36* | 0.29* |

Table 3-1. Nonparametric two-way ANOVA by ranks for the effect of sulphate level and chloride level on phosphate levels (in mg/l) in the soil pore water of the peat and sand soils. Differences are given for three different days. ns= not significant, *=0.05 > P > 0.01 **= 0.01 > P > 0.001

*3.3.2 pH*

The sulphate level affects the pH of the peat soils. When a large quantity of sulphate is added, the pH is lowered. This is the case on the 35$^{th}$ day. Changes in the chloride level also have an effect on the pH in the peat. A raised level of chloride lowers the pH.

The results for the sand soil are somewhat different. In the case of sand the sulphate level does not affect the pH at any time during the experiment. However the chloride level has an effect on the 35$^{th}$ day. That effect is a lower pH when a high chloride level is provided.

| | | PEAT | | | SAND | | |
|---|---|---|---|---|---|---|---|
| | | day 35 | day 54 | day 62 | day 35 | day 54 | day 62 |
| Sulphate level | High | 3.6 | ns | ns | ns | ns | ns |
| | Low | 4.2** | | | | | |
| Chloride level | High | 3.6 | 3.8 | 4.1 | 5.4 | ns | ns |
| | Low | 4.2** | 4.2* | 4.4* | 5.8* | | |

Table 3-2 Nonparametric two-way ANOVA by ranks for the effect of sulphate level and chloride level on pH in the soil pore water of the peat and sand soils. Differences are given for three different days. ns= not significant, *=0.05 > P > 0.01 **= 0.01 > P > 0.001

## 3.4 Plants

### *3.4.1 Ranunculus acris*

The results of this species are shown for the three soil types (table 3-3). Between the soils there are quite some similarities but also some differences.

On clay the light level is affecting the dry weight (a lower weight when a reduced light intensity is provided) and the leaf area ratio and leaf area/leaf weight (both are higher for the reduced light treatment). The length of the shoots is also affected; i.e. the plants grow taller in shaded conditions. When *Ranunculus* is grown on peat the results are somewhat different. The factor light is not causing any differences in dry weight or leaf area ratio. For the light level the results are the same for leaf area/leaf weight and length as for plants on clay. But the full light treatment is causing a higher number of dead plants than when the light level is reduced. On sand the light level is again affecting the leaf area ratio and leaf area/leaf weight (both higher under reduced light levels). Plants grown on sand are also shorter when grown in full light and the mortality is higher under these conditions.

On clay the sulphate level has no effect on *Ranunculus acris*. On peat the sulphate level has some effect. The only parameter affected is the length after 25 days (this is reduced compared to plants grown without extra sulphate). Plants that grow on sand and are exposed to a higher sulphate level are not affected.

The chloride level does have an effect on the dry weight (lower under high chloride level) when plants are grown on clay, the length is reduced when exposed to a high level of chloride and more plants die under those conditions. On peat the chloride level is causing differences in length but this time, plants grown with extra chloride grow taller on the 25$^{th}$ day than plants grown without. This time the extra chloride also raises the number of dead plants. For the sand soil the chloride level has an effect (increasing dry weight when exposed to a high chloride level). Extra chloride is affecting the length after 60 days negatively and also raises the mortality.

| | | DW | LAR | LWR | LA/ LW | LENGTH 25D | LENGTH 60D | MORT 25D | MORT 65D |
|---|---|---|---|---|---|---|---|---|---|
| **Light level** | **Clay** | ** | *** | ns | *** | *** | *** | ns | ns |
| | **Peat** | ns | ns | ns | ** | *** | *** | ns | *** |
| | **Sand** | ns | *** | ns | *** | *** | *** | ns | * |
| | | | | | | | | | |
| **Sulphate level** | **Clay** | ns | ns | ns | ns | ns | ns | ns | ns |
| | **Peat** | ns | ns | ns | ns | ** | ns | ns | ns |
| | **Sand** | ns | ns | ns | ns | ns | ns | ns | ns |
| | | | | | | | | | |
| **Chloride level** | **Clay** | ** | ns | ns | ns | * | * | ns | ** |
| | **Peat** | ns | ns | ns | ns | * | ns | ns | * |
| | **Sand** | ** | ns | ns | ns | ns | * | ns | ** |

Table 3-3. Nonparametric two-way ANOVA by ranks for the effect of light level, sulphate level and chloride level on the different parameters of *Ranunculus acris*. DW=dry weight, LAR=leaf area ratio, LWR=leaf weight ratio, LA/LW=leaf area/leaf weight, length=total length after 25 or 60 days, Mort=number of dead plants after 25 or 65 days. ns= not significant, *=0.05 > P > 0.01 **= 0.01 > P > 0.001 ***= P < 0.001

To determine whether there are differences between the soil types with regard to the parameters, the results for all soil types were put together and tested again (table 3-4).

| | DW | LAR | LWR | LA/ LW | LENGTH 25D | LENGTH 60D | MORT 25D | MORT 65D |
|---|---|---|---|---|---|---|---|---|
| **Soil type** | *** | *** | ns | ** | *** | *** | ns | *** |

Table 3-4. Nonparametric two-way ANOVA by ranks for the effect of soil type on the parameters of *Ranunculus acris*. For abbreviations see table 3-3.

The different soils used to grow the plants had also a significant effect on most of the parameters. The total dry weight of the plants is the lowest on peat, somewhat higher on sand and the highest on clay. The soil type also affects the leaf area ratio, in this case the peat shows the lowest ratio. The same result is found for the leaf area/leaf weight. The length after 25 days has some different results. Plants grown on peat are the smallest, plants grown on clay grow taller, but the plants on sand perform best. After 60 days the difference in length between sand and clay is no longer measurable. The mortality at last is the highest on peat and sand and the lowest on clay.

*3.4.2 Sanguisorba officinalis*

The effects described for *Ranunculus acris* do also seem to be valid for *Sanguisorba officinalis*, with some slight differences.

On clay the light level has the most effect of the three stress factors. It is affecting the dry weight (lower for plants grown in shade) and both leaf area ratio and leaf area/leaf weight (both higher for plants grown in shade). And again the plants grown under the reduced light level are longer than the plants grown in full light. The factor light also affects the plants grown on peat. The lower light level results in higher values for the leaf area ratio and the leaf area/leaf weight. These plants do also grow taller than the plants grown in full light. On sand the results are similar for the leaf area ratio and the leaf area/leaf weight when the light level is changed.

The sulphate level has no effect on any of the parameters.

The higher chloride level does have a negative effect on the dry weight and on the leaf weight ratio of plants grown on clay. The only difference with the results for the clay and the peat soils is that plants grown on sand exposed to a high level of chloride show a higher mortality.

| | | DW | LAR | LWR | LA/ LW | LENGTH 25D | LENGTH 60D | MORT 25D | MORT 65D |
|---|---|---|---|---|---|---|---|---|---|
| **Light Level** | **Clay** | ** | ** | ns | *** | *** | *** | ns | ns |
| | **Peat** | ns | *** | ns | *** | ns | *** | ns | ns |
| | **Sand** | ns | *** | ns | *** | ns | *** | ns | ns |
| | | | | | | | | | |
| **Sulphate level** | **Clay** | ns | ns | ns | ns | ns | ns | ns | ns |
| | **Peat** | ns | ns | ns | ns | ns | ns | ns | ns |
| | **Sand** | ns | ns | ns | ns | ns | ns | ns | ns |
| | | | | | | | | | |
| **Chloride level** | **Clay** | * | ns | * | ns | ns | ns | ns | ns |
| | **Peat** | ns | ns | ns | ns | ns | ns | ns | ns |
| | **Sand** | ns | ns | ns | ns | ns | ns | ns | * |

Table 3-5. Nonparametric two-way ANOVA by ranks for the effect of light level, sulphate level and chloride level on the different parameters of *Sanguisorba officinalis*. For abbreviations see table 3-3.

Combining the soils again gives a clearer image of the total effect of the soil type. This table (table 3-6) differs considerably from the one of *Ranunculus* (table 3-4).

| | DW | LAR | LWR | LA/ LW | LENGTH 25D | LENGTH 60D | MORT 25D | MORT 65D |
|---|---|---|---|---|---|---|---|---|
| **Soil type** | ns | ns | ns | ns | *** | ns | ns | ns |

Table 3-6. Nonparametric two-way ANOVA by ranks for the effect of soil type on the parameters of *Sanguisorba officinalis*. For abbreviations see table 3-3.

The impact of the different soil types on the growth of *Sanguisorba officinalis* is less extreme than for *Ranunculus acris*. The soil type had only a significant effect on the length of the plants after 25 days.
Plants grown on peat or sand show no differences but plants grown on clay perform worse.

## 4. Discussion and conclusions

### The experimental set-up

Some of the used methods did not always have the desired effect. The soils did not become anoxic, which prevented sulphide production. This may be caused by the water level that was not high enough, but also the fact that the plastic pipes were placed in a container may have worked adversely. Because the containers were not covered, the water surface was exposed to the air in the climate rooms and exchange between air and water might have prevented oxygen depletion. These problems may be overcome in future research by sealing the topside of the containers and possibly those of the PVC-pipes to. It may also be possible to grow the plants in a container filled with soil and waterlogging that soil without using pumps, i.e. without water flow. That would also prevent the supply of oxygen rich water. Another explanation for the fact that there was still oxygen available in the soil could be that these species are capable of Radial Oxygen Loss (ROL). In this process the roots release oxygen into the soil. It is not known if *Sanguisorba officinalis* and *Ranunculus acris* are capable of ROL however.

The climate room in which the light was filtered experienced some serious technical problems. During the experiment this climate room showed some irregularities. In the first place the humidity; the control panel gave some values that were probably not representative for the actual situation. The cooling system was also malfunctioning, causing the temperature to be one or two degrees higher as intended. This malfunction resulted in a serious problem in the sixth week of the experiment in which the temperature increased to 24°C. After the reparation no further problems with regard to the temperature arose.

Using pumps also proved to be a factor, which caused some variation. For every six containers one pump was used. Each container had it's own tubing running through the pump. Each of these had to be adjusted so that the pumped amount was the same for every container. However some tubes became slightly clogged by growth of algae, which prevented proper flow of the water. And in some cases the pressure inside the pump was uneven.

Although not apparent in the results, the clay soil was also causing some difficulties. Each soil was meant to become anoxic by capillary rise of the water. In the clay soil this capillarity proved difficult. This was due to the fact that part of the water in the soil cores had already evaporated, compacting the soil structure. The cores had to be pierced to make the water level rise. The peat and the sand soil retained their capillary rise.

Another thing that needs improvement is the fact that after a while some plants reach a height at which they are covering other plants. Especially the plants grown in the shade developed long stems and their leaves overlapped other plants. This is a serious problem because it affects the objective of the experiment in which each plant has to encounter the same light level. It would be better to grow plants with a greater distance in between.

Finally the chloride level in the containers did not correspond to the level that was calculated in advance. This could be caused by a miscalculation during the production of the solutions, or the extra chloride was released from the soils.

### Light level

The first feature in the results of *Ranunculus acris* is that changing the light level affects different parameters than when the sulphate- and chloride levels are changed. On clay the lower light intensity resulted in a higher value of leaf area/leaf weight and the leaf area ratio. Another feature, which is quite obvious, is the fact that the plants grow taller when exposed to a lower light level. The effects on peat are somewhat different. Although not affecting parameters such as the dry weight or the leaf area ratio the factor 'light' does affect the mortality number of *Ranunculus*. Strangely the plants on peat grown in the full light treatment show a higher number of dead than the plants grown in the shaded treatment. This might be due to the action of micro-organisms living on or just below the surface of that peat soils, *Sanguisorba officinalis* does not show this effect however. For sand the results do not differ much from those on clay, but this time the light level also affects the mortality.

The results for *Sanguisorba officinalis* are slightly different. The light level affects the parameters involving the leaves. The lower light level results in higher values of the leaf area ratio and leaf weight ratio. The mortality is not affected and the biomass production only on clay.
Reducing the light level did not always affect the biomass production and it affected the mortality both negatively and positively. Therefore it is difficult to refer to the reduced light level as a stress factor in the present experiment.
This experiment had some results that were in accordance with the expectations, but there were also some unexpected effects. Reducing the light intensity for instance did not affect the aboveground dry weight of both plant species. This is in contrast with previous work (among others Kotowski *et al.* 2001). The explanation might be that the roots were not used in the analyses. When these dry weights were added to those of the shoots, the total dry weight might have been significant different. The lower light level stimulated the plants to invest in their leaves. This is reflected in the way the leaf area ratio and the leaf area/leaf weight increase. This result is in accordance with that of Kotowski *et al.* (2001) and Anten & Hirose (1999). However a result the latter achieved was the fact that plants when grown under shady conditions have to allocate biomass to the stems. This way they can produce longer stems to escape from the shadow, but these stems do also have to have a more solid structure. The consequence of this allocation is that the weight investment in the leaves decreases. In the present experiment however the leaf weight ratios did not decrease when the plants were exposed to a reduced light level. The plants did nevertheless produce longer stems. Again the fact that the root weight is not taken into account is making it difficult to be certain about the way the plant is allocating its biomass.

**Sulphate level**
To test the effect of sulphide on both plant species a high sulphate level was combined with a high water level. However the measurements reveal that the oxygen saturation of the soil did not decrease to zero. Soils may be oxygen depleted when the redox potential reaches values lower than ca. 330 mV (Scheffer & Schachschabel 1984). But in the present experiment the redox measurements indicate that the redox potential was not at a level at which sulphide production takes place, but at the level at which nitrate is reduced. In short; sulphide as a stress factor did probably not occur in this experiment
The pH does also give information on the processes taking place in the soil cores. In anoxic soils the subsequent redox reactions do affect the redoxpotential but also the pH. In moderate acid soils (such as the soils in the experiment) the pH rises to neutral values, because the $H^+$ ions are used in the redox reactions. Although the pH does not rise between the different days of sampling the pH of the sand soil seems to have a higher value than before the start of the experiment (see table 2-1). This rise may be caused the reduction reactions but also the solutions that were given can be responsible, because these had also a higher pH than the soils.
Even though the fact that no sulphide was produced, the sulphate level did play a role in influencing the plant growth. It did affect the length of *Ranunculus acris* after 25 days (perhaps the ionic concentration of sulphate was toxic for the seedling at that moment), but in the end these differences were no longer evident. For *Sanguisorba officinalis* the level of sulphate has no effect at all.

**Chloride level**
Changing the chloride level to a higher concentration did result in less biomass of *Ranunculus acris* and the final length was also decreased. And because it has a highly significant effect on the mortality, this factor can indeed be called a stress factor.
The high level of chloride did affect the growth of *Sanguisorba* as it did for *Ranunculus*. It had a negative effect on the dry weight and the overall effect was a higher number of dead plants. Just as for *Ranunculus acris* the high chloride level acts as a stress factor.

**Soil effect**

To relate the conditions in this experiment to the project area of Midden-Groningen three soil types were used: clay, peat and sand. The soil type was not introduced as a stress factor but all in all proved to be a differentiating one. *Ranunculus acris* seems to favour clay over sand and peat. Besides affecting the total shoot biomass the soil type also affects the leaf area ratio, the final length and the number of dead plants. On the peat and sand soils the number of dead plants was very high (±40%). In literature this preference for clay is not so profound (Van der Meijden 1996, Weeda *et al.* 1985). An explanation might be that *Ranunculus acris* responded negatively to the low pH in the sand and the peat soil. Processes involved in the nitrogen cycle in the soils might also cause differences. Mineralisation, nitrification and denitrification are all affected by (among other things) the oxygen content. But except from the initial measurement of the nitrogen content, such soil analyses have not been performed later in the experiment. Therefore it is difficult to make decisions on the soil specific differences on that point.

The other plant, *Sanguisorba officinalis* did not show different responses for the different soil types. The only parameter that is affected is the length after 25 days. The plants have a preference for peat and sand. But after 65 days the differences are no longer existent, so *Sanguisorba officinalis* does not seem to be affected by this factor after all. This is in accordance to literature that this plant can be found on mixed soils; sandy clay to fen soils (Van der Meijden 1996). So with regard to the soil type the both plant species should have been more or less comparable.

**Internal eutrophication**

In the experiment the plants were exposed to considerable amounts of sulphate and chloride. Though these factors were introduced as stress producers, they can also act in a way that affects plants positively. This process is described as internal eutrophication. As mentioned before three processes are responsible for this process. First of all no sulphide is produced. Therefore this mechanism can not be responsible for the release of phosphate in the present experiment. In the experiment the analysis shows that the phosphate availability does not increase when sulphate or chloride is supplied. The opposite happens: in peat and in sand the phosphate level decreases. It's difficult to be decisive on this point, because the release of phosphate may be depending on the iron/phosphate ratio in the sediment pore water (Smolders *et al.* 2001). Also associated to internal eutrophication is an increasing pH. Usually this is due to bicarbonate enrichment by supply water. In this experiment water of the public water supply has been used, which contains some bicarbonate but in none of the cases the pH increased. This means that internal eutrophication did probably not occur. It would be interesting to measure the nitrogen and phosphorus content of the plants. This could answer the question whether there was more phosphate taken up when sulphate or chloride was added to the soil.

The ratio between nitrogen and phosphorous in the plant biomass could also answer the question why the plants in the different treatments responded as they did. It is possible that the plant species experienced a deficiency for nitrogen and/or phosphorous (Koerselman & Meuleman 1996, Pegtel *et al.* 1996).

**Conclusions**

The first question was: Does the rare plant species *Sanguisorba officinalis* suffer more from the stress factors than the common species, *Ranunculus acris*? This hypothesis has to be rejected. For most cases *Ranunculus acris* responded with a larger reduction in biomass or more dead plants than *Sanguisorba officinalis*. It is also more selective with regard to the soil type than *Sanguisorba officinalis*. The latter shows a capability of coping with the conditions it's exposed to. Concluding: In this case the rare species suffers less from the stress factors than the common species.

A second hypothesis was that competition for light would be more important in determining the successful settlement of a plant than stress by sulphide and/or chloride. As the production of sulphide did (probably) not occur, this factor cannot be used in the comparison. The light level and the chloride level remain. In this experiment the results indicate that stress by a high chloride level (10.6 mmol/l) has more effect than competition for light. The light level did only affect the length of the plants and the leaf characteristics. It had no effect on the survival or on the biomass production. For this experiment the hypothesis has to be rejected.

As mentioned before the difference in 'rareness' between these species could be caused by differences in stress tolerance, in dispersal or both. Both the biotic tolerance (competition for light) and the abiotic tolerance (exposal to high levels of sulphate and chloride) have been examined. These treatments did not show any differences in tolerances that may cause *Sanguisorba officinalis* to be rare and *Ranunculus acris* to be common. That means that for these two species the differences are caused by something else. This could be the stress tolerance in other life stages, the tolerance towards other stress factors or differences in dispersal between the two species. Though not much knowledge is available on this topic (at least not for *Sanguisorba officinalis*) it seems that *Ranunculus acris* has better chances for dispersal. The last species has a lower number of seeds per plant than the first but the seeds have a higher weight. This can be a positive and a negative trait. Seedlings from heavier seeds might have better chances for germination because they have more nutrition from that seed, but bigger seeds do also run a higher risk of being damaged when eaten by cattle. Not many data are available on the distance of dispersal but it seems that because *Sanguisorba* grows in wetter habitats than *Ranunculus acris* does, the chances of dispersal by cattle or by human activity are smaller than for *Ranunculus* (Grime 1988, Van der Meijden 1996, pers. comm. René Bekker).

The third question was: Do the imposed stress factors potentially limit establishment of target species? For sulphide this question cannot be answered. Changing the light level did not affect the plant growth. In the present experiment the light level doesn't have an effect.
The chloride level has a very strong impact on the plant growth in this experiment. This means that *Ranunculus acris* and *Sanguisorba officinalis* will encounter serious problems in establishing in area with similar chloride levels. Especially in the area of the reserve in Midden-Groningen this will be relevant because large parts do show high levels of chloride.

**Recommendations**

- First of all the effect of sulphide is a factor that needs further research. The experimental design was not useful to produce sulphide and examine its consequences. The improvements involving the oxygen content of the soils have been mentioned before.
- For *Ranunculus acris* and *Sanguisorba officinalis* the present experiment does not explain why the first is quite common and the other rare. More data on their dispersal could be valuable in clearing this question. That means more research for instance of their seed number, seed morphology, ways of dispersal and the distance of dispersal.
  Another experiment could involve testing their tolerance towards other (stress) factors, for instance changes in the ground water level or the influence of mowing (because the *Fritillario alopecuretum pratensis* is a hay meadow).
  For these species a third possible research could be to test their tolerance to a range of factors (including chloride and sulphide) during different life stages, because in the present experiment only seedlings were used.

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## Appendix 1 The experimental set-up

Data on the experimental set-up

Table a-1. Information on the pump and the tubing

| | |
|---|---|
| **Tubing** | MASTERFLEX® NORPRENE® L/S13 & VEPRENE® L/S 13 |
| **Pump cartridge** | MASTERFLEX® 7519-65 |
| **Pump motor** | MASTERFLEX® 7521-57 |
| **Pump head** | MASTERFLEX® 7519-25 |

Table a-2. Composition of the solutions

| **Chloride solution** | | **Sulphate solution** | |
|---|---|---|---|
| | Concentration (mg/l) | | Concentration (mg/l) |
| KCl | 126.82 | $K_2SO_4$ | 87.15 |
| NaCl | 157.95 | $Na_2SO_4$ | 71.05 |
| $MgCl_2+6H_2O$ | 223.63 | $MgSO_4$ | 60.10 |
| $CaCl_2$ | 66.66 | $CaSO_4+2H_2O$ | 86.10 |

Table a-3. Information on the harvesting period and place of the seeds of the used plants.

| | **Harvesting period** | **Location** | **Germinationpercentage** |
|---|---|---|---|
| **Sanguisorba officinalis** | August 2002 | De Reest, Meppel The Netherlands | 72% |
| **Ranunculus acris** | ? -2002 | France | 54% |

Table a-4. Specifications of the soil types used in the experiment

| SOIL TYPE | COLLECTING DATE |
|---|---|
| Clay | 4&10-10-2002 |
| Peat | 4-10-2002 |
| Sand | 16-10-2002 |

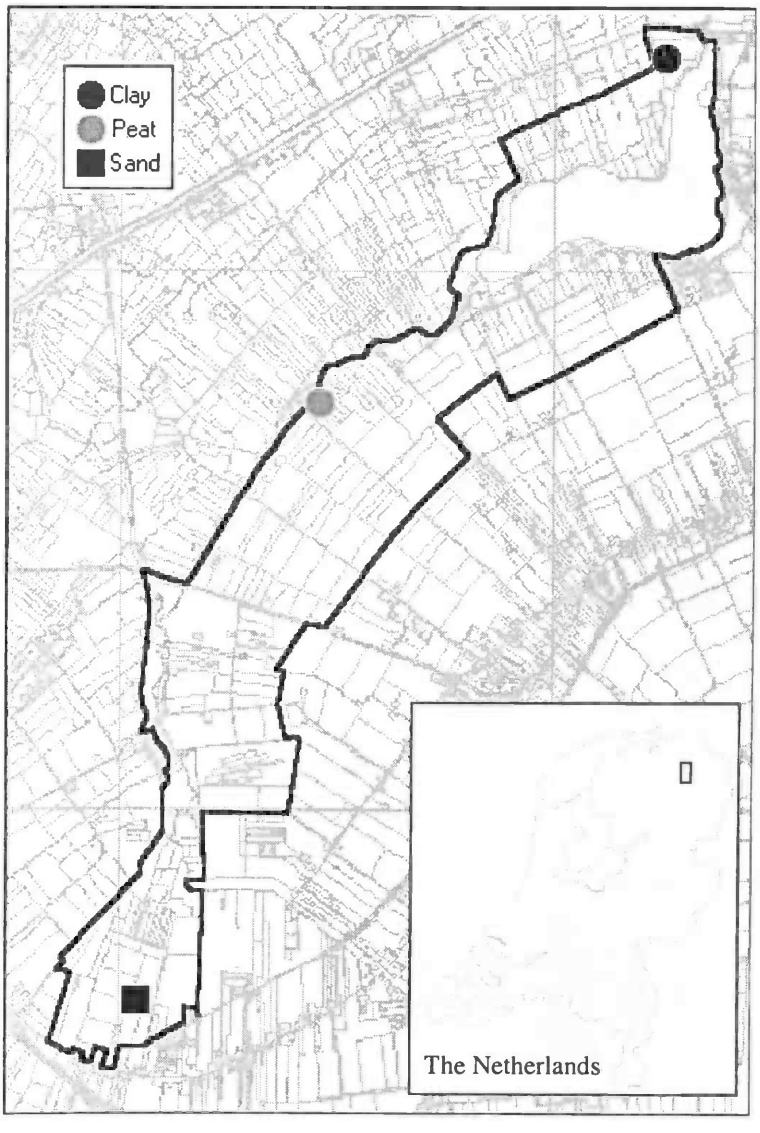
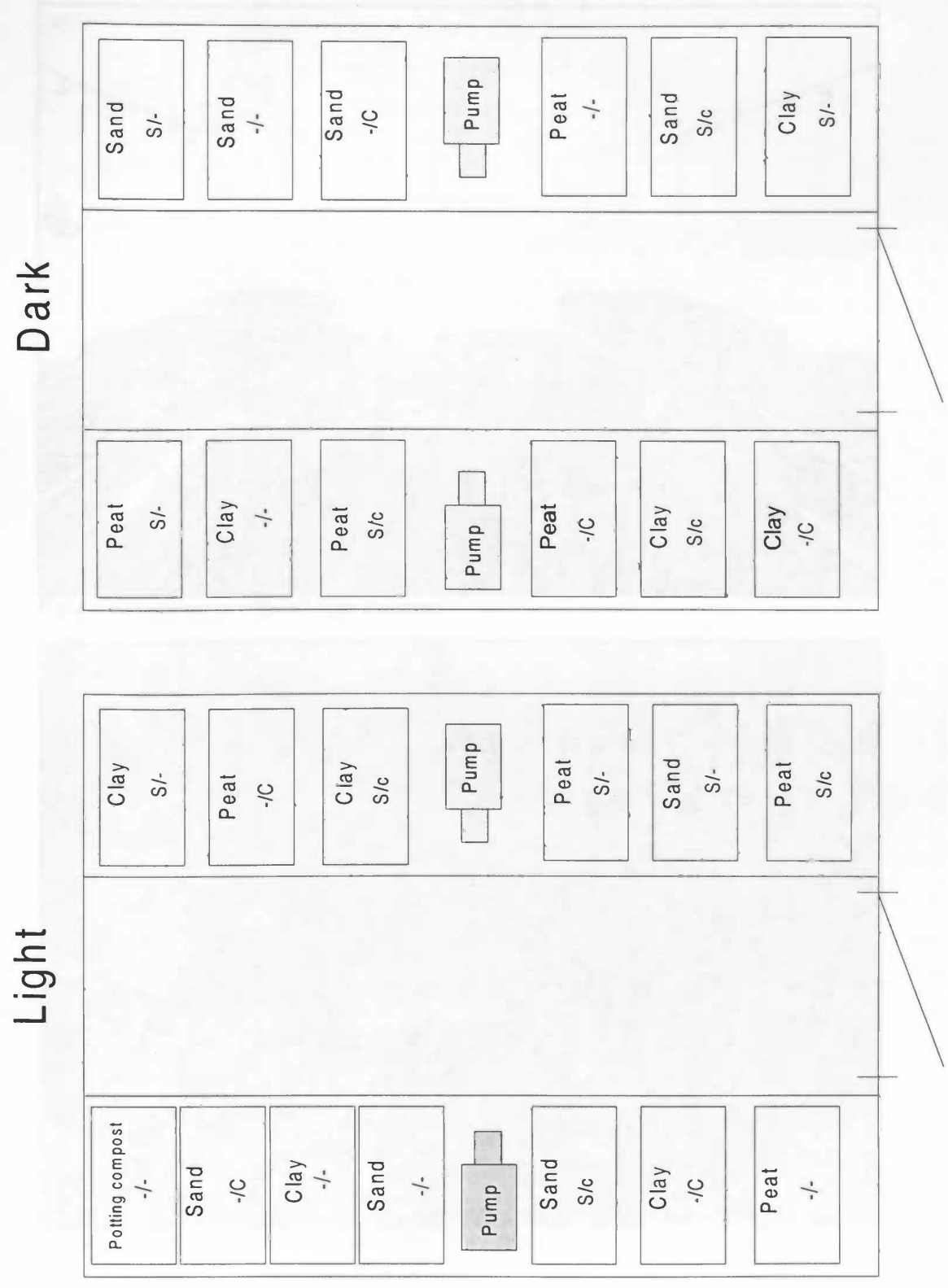



Fig a-1. Locations in the area of Midden-Groningen where the soil cores for the experiment were collected. (For detailed descriptions of the project area see Van Diggelen *et al.* 2000)

Figure a-2. The experimental set-up in the climate rooms.



Pictures of the climate rooms

Fig a-3. Climate room with full light treatment

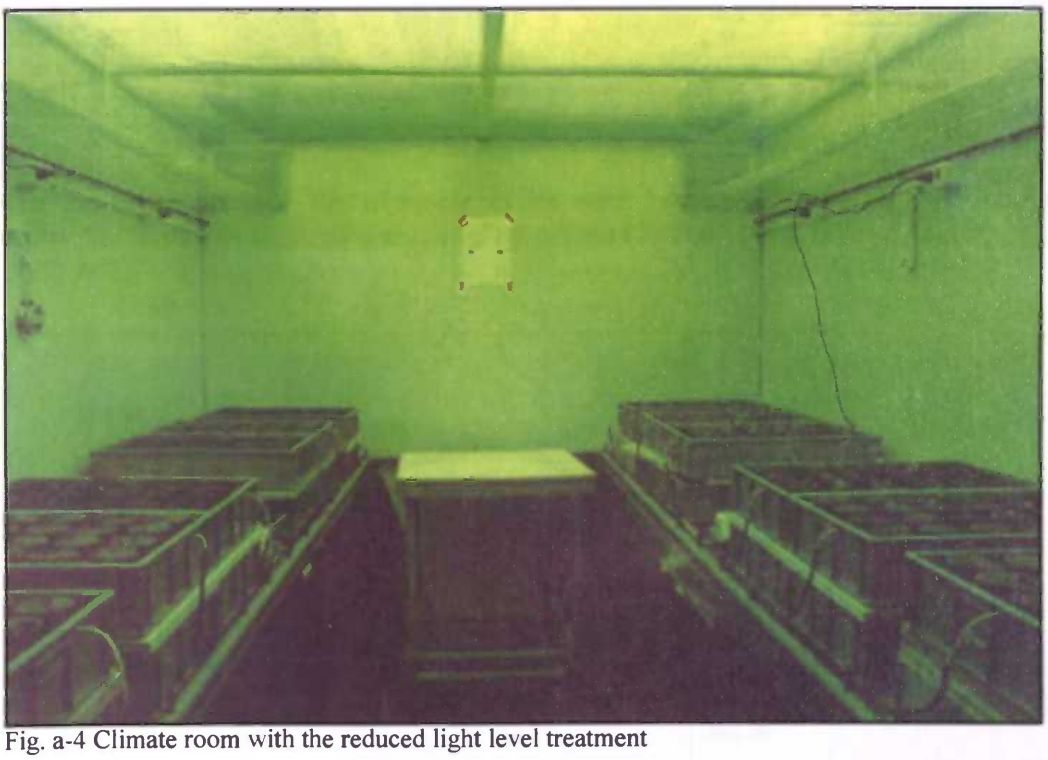


Fig. a-4 Climate room with the reduced light level treatment

## Appendix 2 Analysis

Methods used for characterising the three soil types, which are used in the experiment.

Moist content
By grinding, each soil core was homogenised. Samples of the soil cores were then weighed and dried overnight at 105°C. The moist content could be calculated after weighing the dried soil samples again.

Organic matter
The dried (at 105°C) soil samples were dried at 500°C. The samples were then weighed again and further calculation resulted in the organic matter content of the three soil types. The samples were again dried at 850°C, which made sure that all the $CO_2$ was removed. Finally the soil samples were weighed again.

The remainders of the soil cores not used in the methods were stored dry at 35°C.

pH
The pH of each soil type was determined in tubes in which a soil sample was added to destillated water. The results of these measurement is called the pH($H_2O$). The pH(KCl) was measured in the same tubes but 1M KCl was added.

Total N-content
To determine the total N-content of each soil type the Kjeldahl-Lauro method was used.

Total P-content
The total P-content of the experimental soils was determined with al molybdenum colouring after destruction of each soil sample.

Micro-electrodes
The measurements with the micro-electrodes were carried out following the instructions written by Henk van Gemerden also used by Adema (1997).

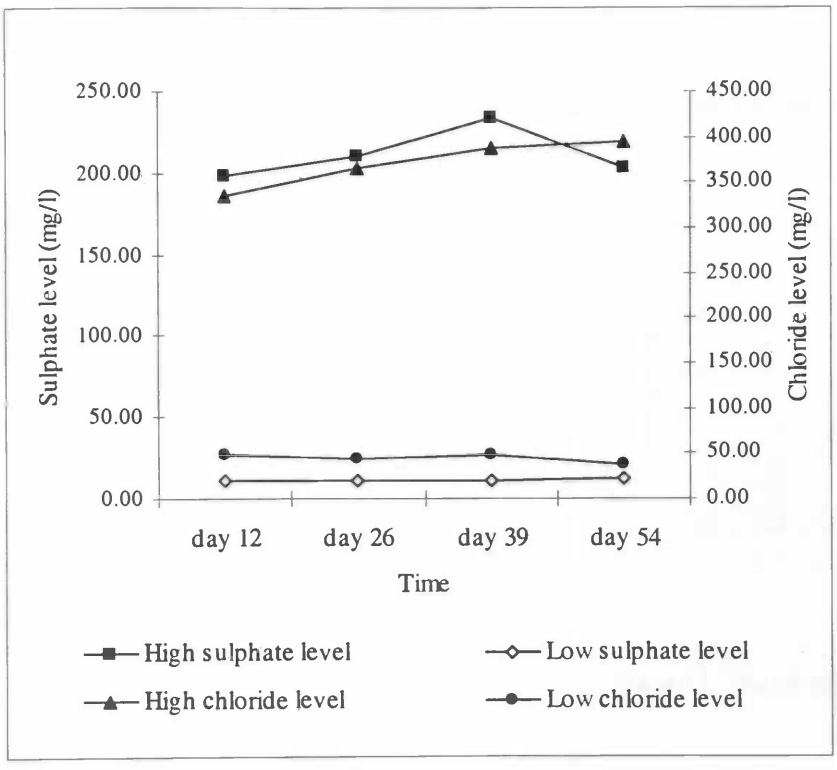



Fig a-5. The sulphate- and chloride levels in the containers of the different treatments.

**Appendix 3 Soils**

The phosphate levels in the pore water of the peat and the sand soils at different times during the experiment. Error bars are SE of means.

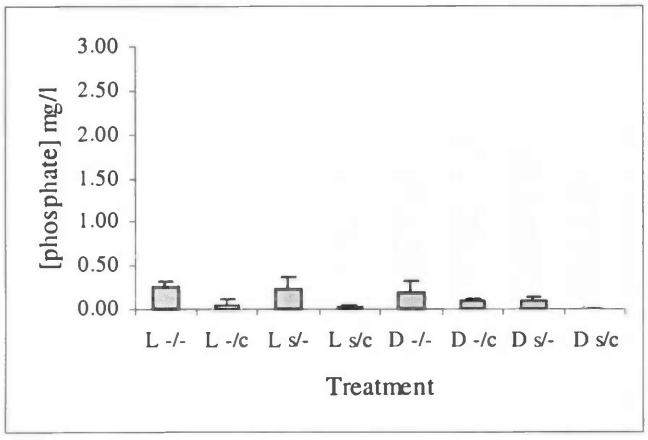



Fig. a-6. The phosphate level of the peat soil at day 35.

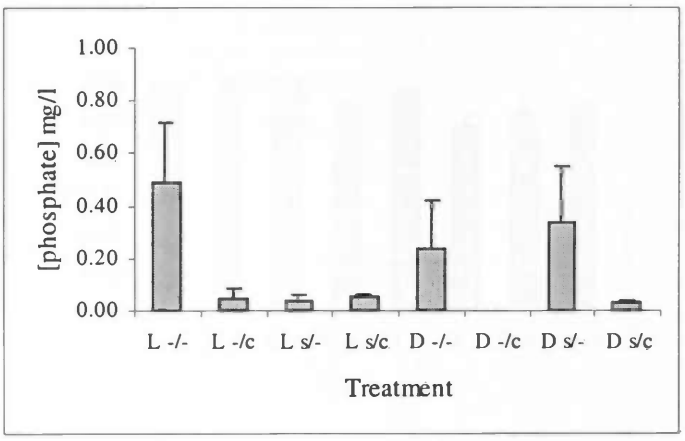



Fig. a-9. The phosphate level of the sand soil at day 35.

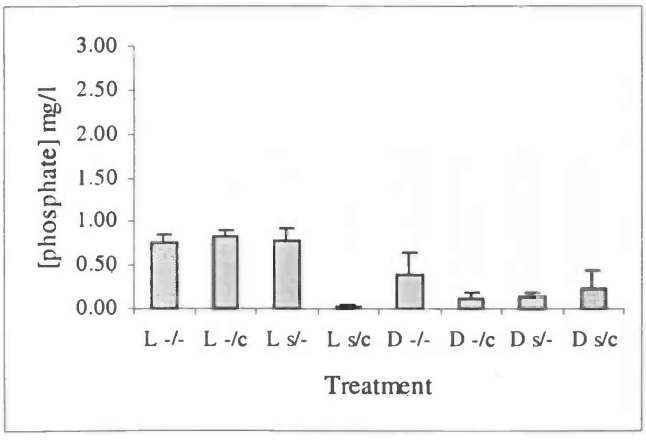



Fig. a-7. The phosphate level of the peat soil at day 54.

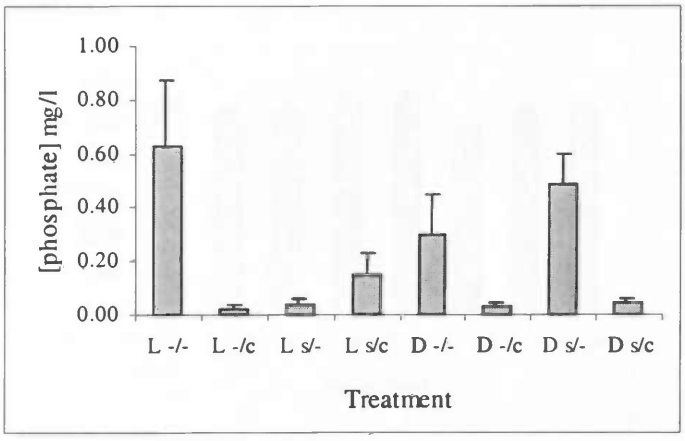



Fig. a-10. The phosphate level of the sand soil at day 54.

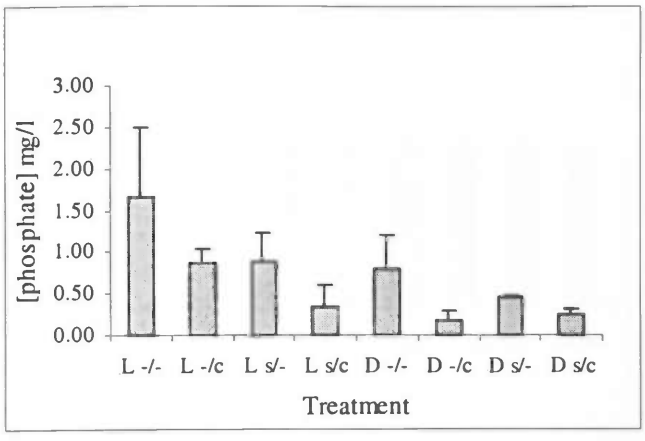



Fig. a-8. The phosphate level of the peat soil at day 62.

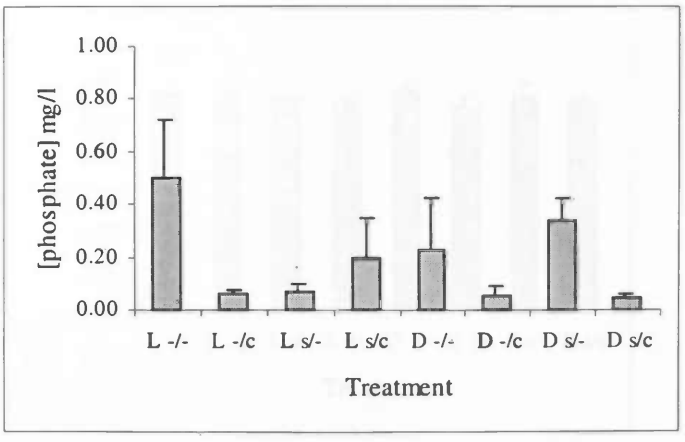



Fig. a-11. The phosphate level of the sand soil at day 62.

The pH in the pore water of the peat and the sand soils at different times during the experiment. Error bars are SE of means.

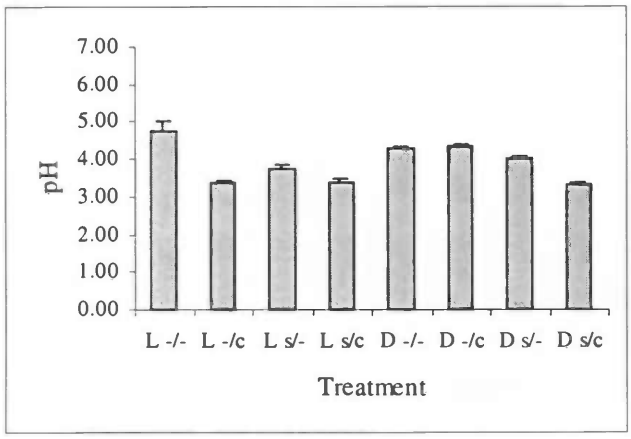



Fig. a-12. The pH of the peat soil at day 35.

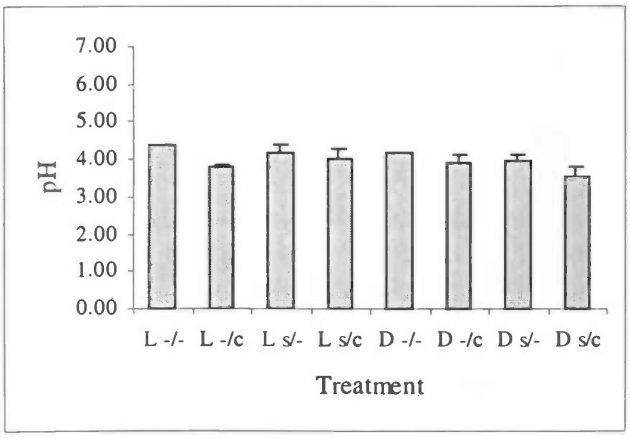



Fig. a-13. The pH of the peat soil at day 54.

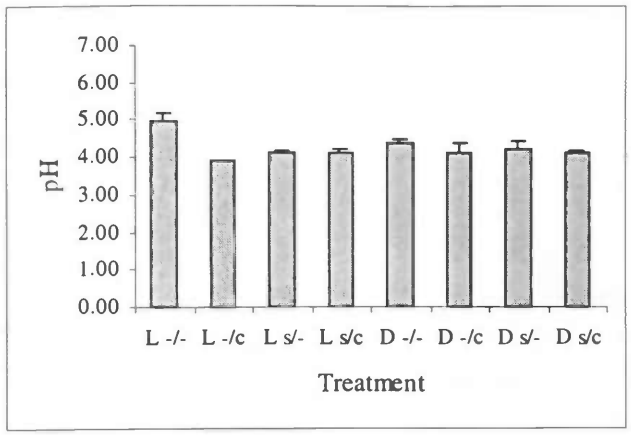



Fig. a-14. The pH of the peat soil at day 62.

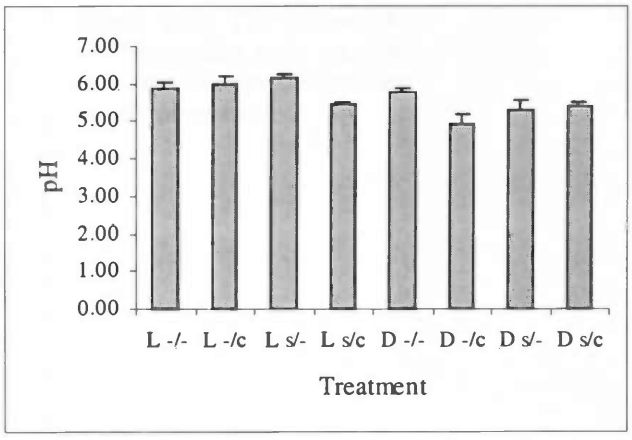



Fig. a-15. The pH of the sand soil at day 35.

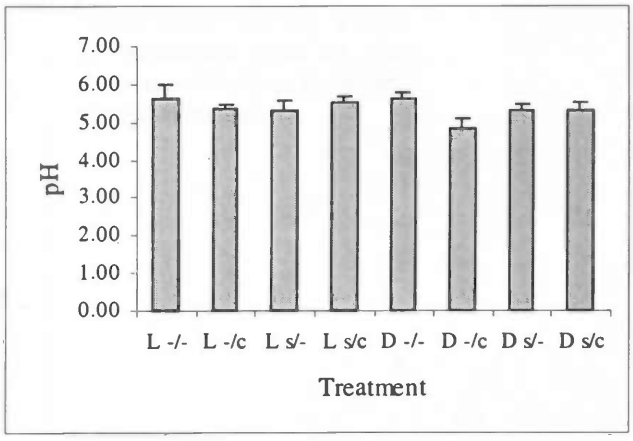



Fig. a-16. The pH of the sand soil at day 54.

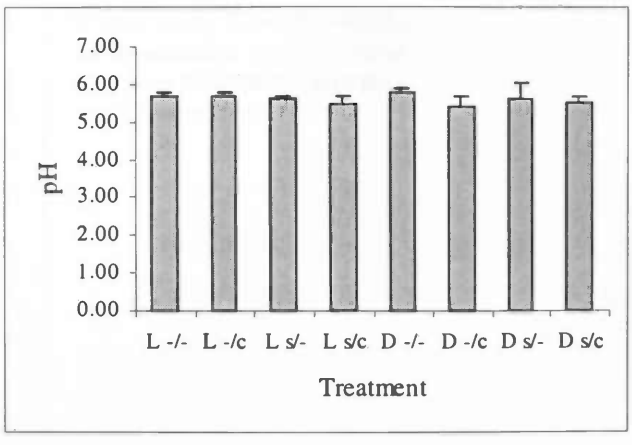



Fig. a-17. The pH of the sand soil at day 62.

## Appendix 4 Plants

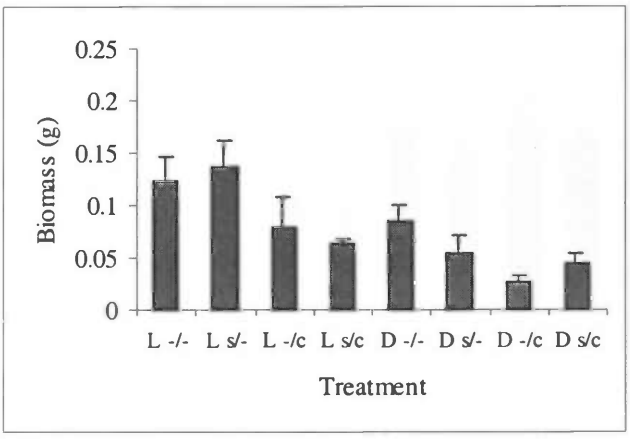



Figure a-18. The dry weight of *Ranunculus acris* on clay for every treatment. Error bars are SE of means.

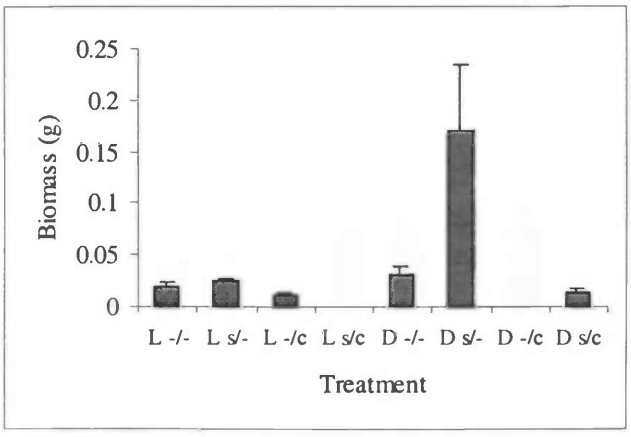



Figure a-19. The dry weight of *Ranunculus acris* on peat for every treatment. Error bars are SE of means.

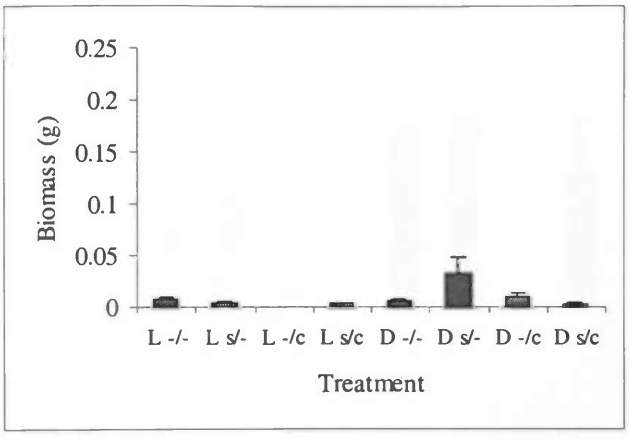



Figure a-20. The dry weight of *Ranunculus acris* on sand for every treatment. Error bars are SE of means.

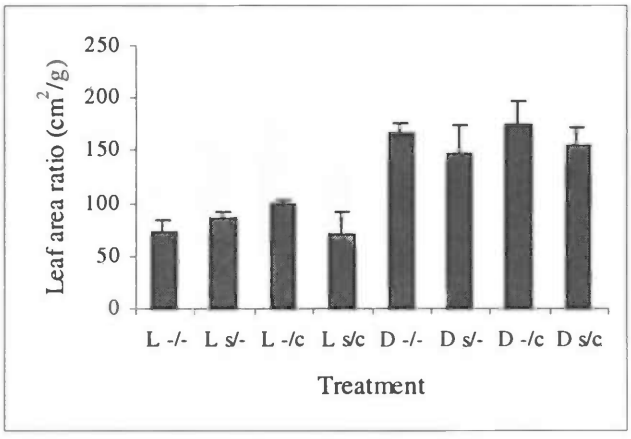



Figure a-21. The leaf area ratio of *Ranunculus acris* on clay for every treatment. Error bars are SE of means.

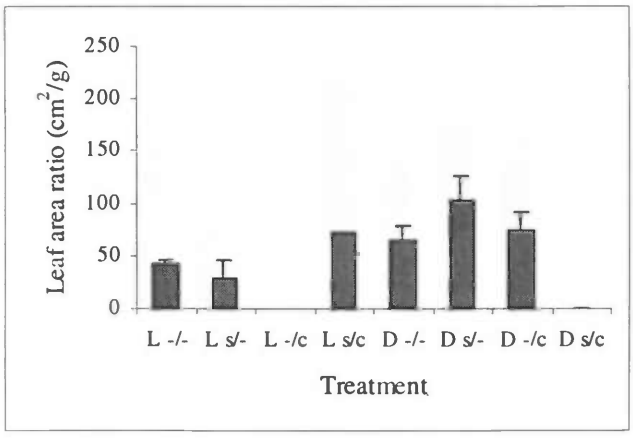



Figure a-22. The leaf area ratio of *Ranunculus acris* on peat for every treatment. Error bars are SE of means.

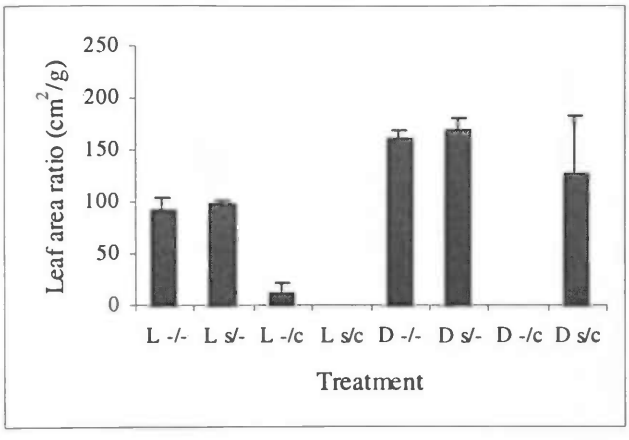



Figure a-23. The leaf area ratio of *Ranunculus acris* on sand for every treatment. Error bars are SE of means.

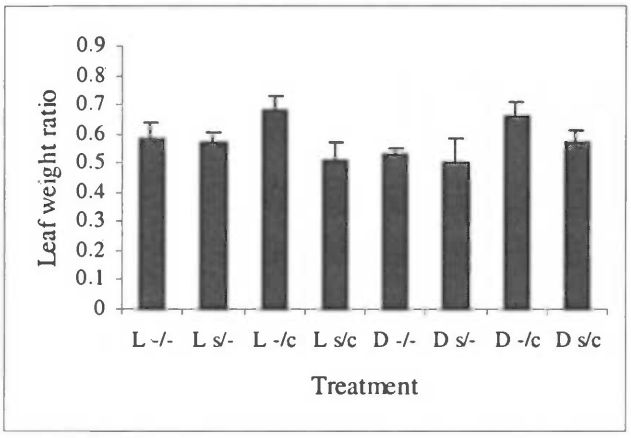



Figure a-24. The leaf weight ratio of *Ranunculus acris* on clay for every treatment. Error bars are SE of means.

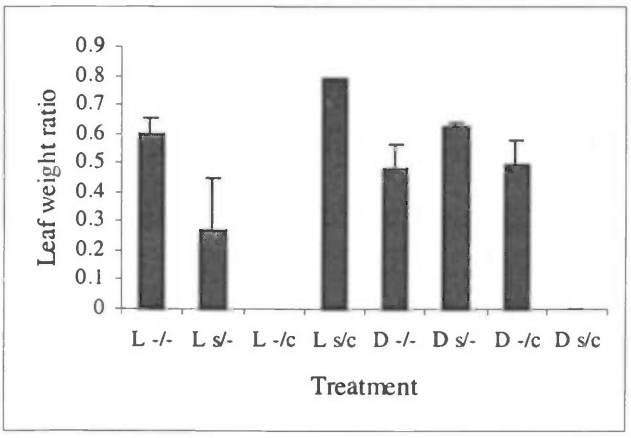



Figure a-25. The leaf weight ratio of *Ranunculus acris* on peat for every treatment. Error bars are SE of means.

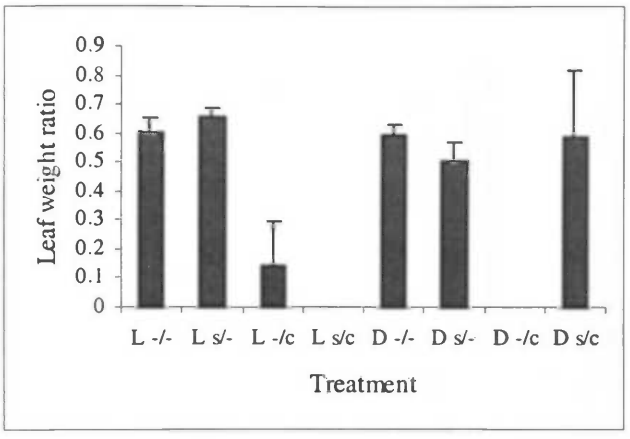



Figure a-26. The leaf weight ratio of *Ranunculus acris* on sand for every treatment. Error bars are SE of means.

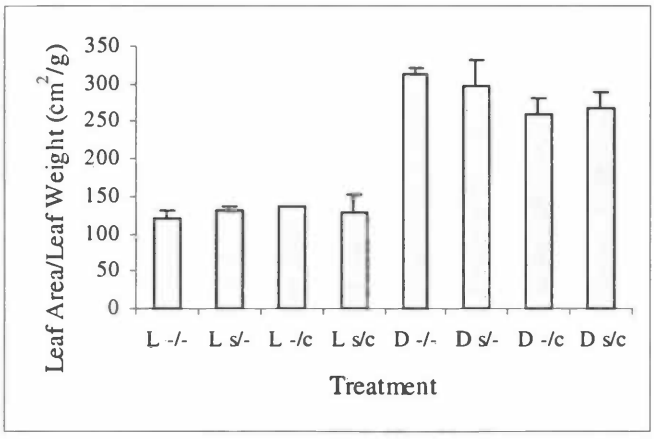



Figure a-27. The leaf area/leaf weight of *Ranunculus acris* on clay for every treatment. Error bars are SE of means.

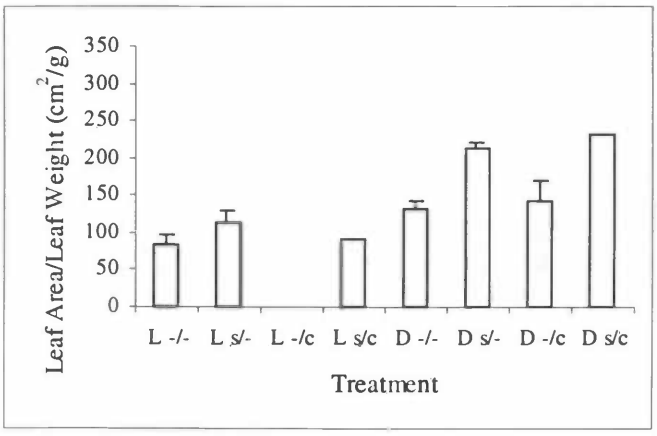



Figure a-28. The leaf area/leaf weight of *Ranunculus acris* on peat for every treatment. Error bars are SE of means.

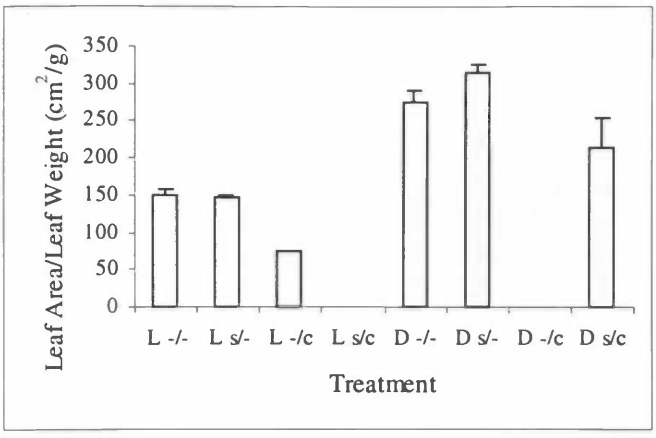



Figure a-29. The leaf area/leaf weight of *Ranunculus acris* on sand for every treatment. Error bars are SE of means.

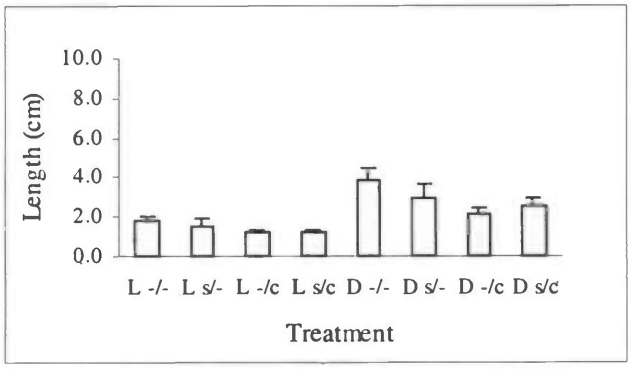



Figure a-30. The length (after 25 days) of *Ranunculus acris* on clay for every treatment. Error bars are SE of means.

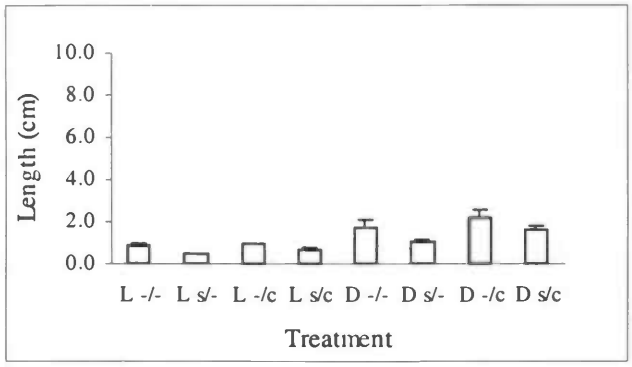



Figure a-31. The length (after 25 days) of *Ranunculus acris* on peat for every treatment. Error bars are SE of means.

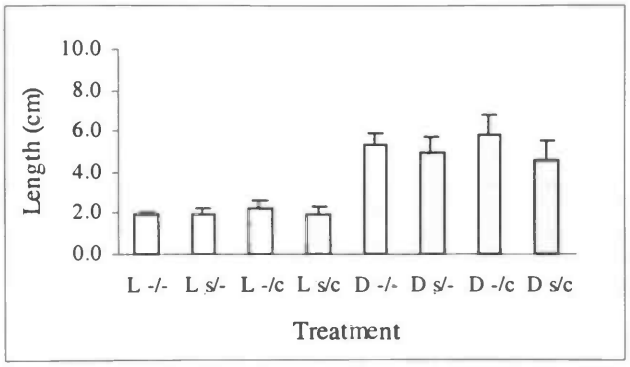



Figure a-32. The length (after 25 days) of *Ranunculus acris* on sand for every treatment. Error bars are SE of means.

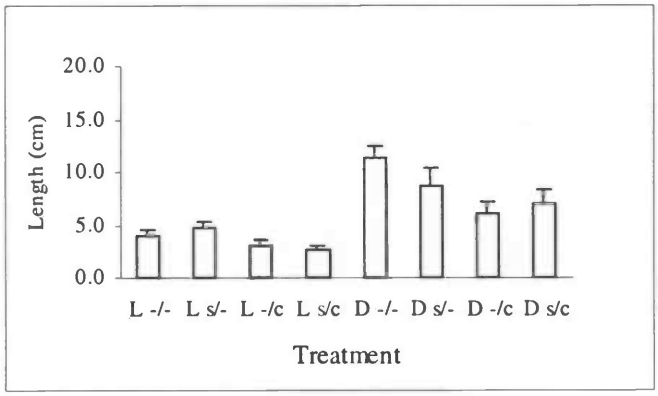



Figure a-33. The length (after 60 days) of *Ranunculus acris* on clay for every treatment. Error bars are SE of means.

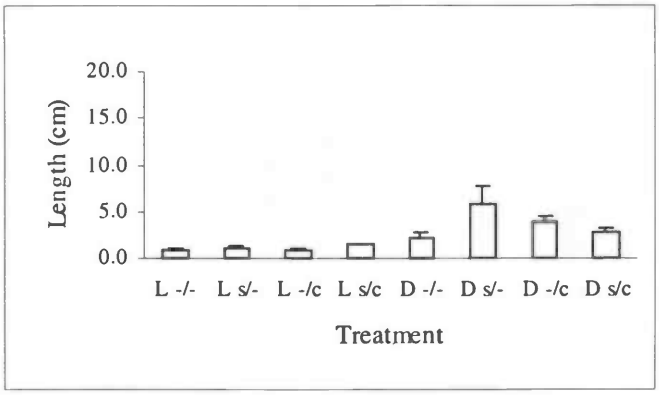



Figure a-34. The length (after 60 days) of *Ranunculus acris* on peat for every treatment. Error bars are SE of means.

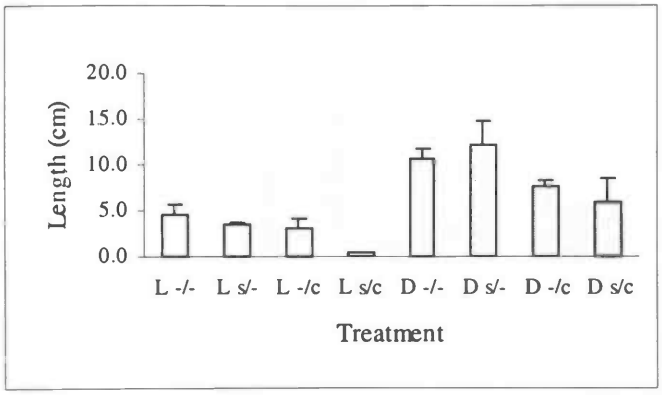



Figure a-35. The length (after 60 days) of *Ranunculus acris* on sand for every treatment. Error bars are SE of means.

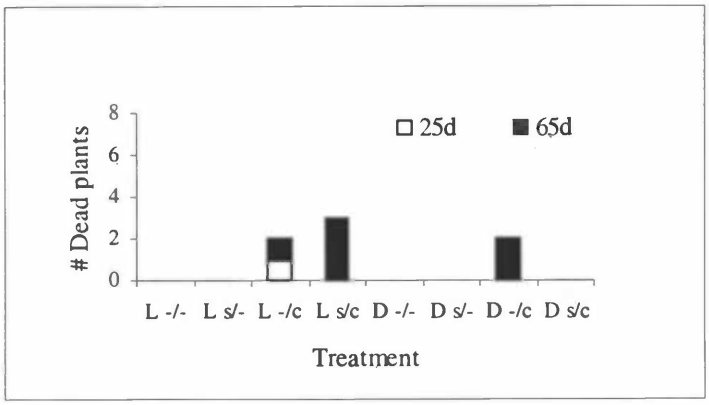



Figure a-36. The mortality (after 25 and 65 days) of *Ranunculus acris* on clay for every treatment.

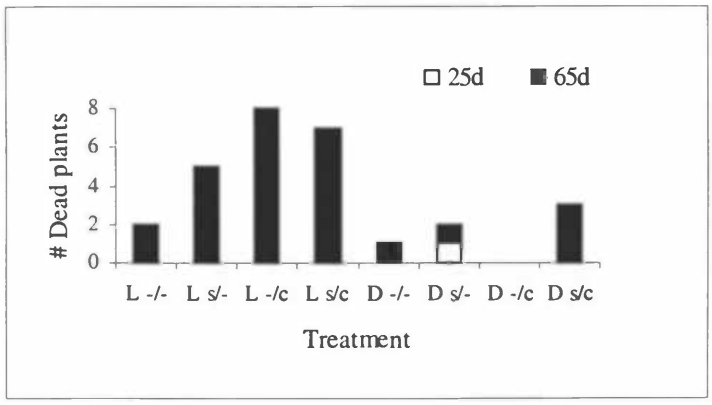



Figure a-37. The mortality (after 25 and 65 days) of *Ranunculus acris* on peat for every treatment.

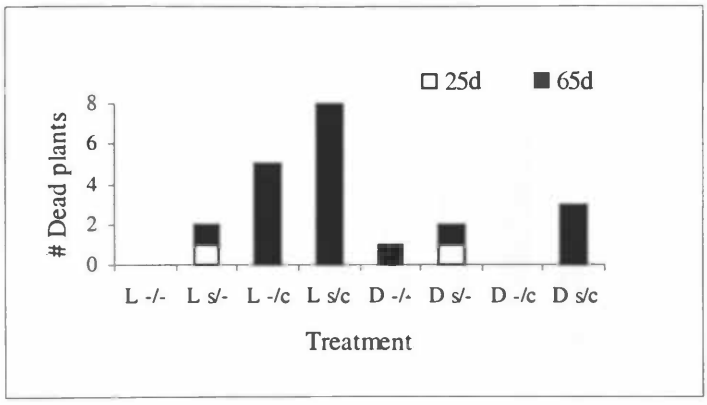



Figure a-38. The mortality (after 25 and 65 days) of *Ranunculus acris* on sand for every treatment.

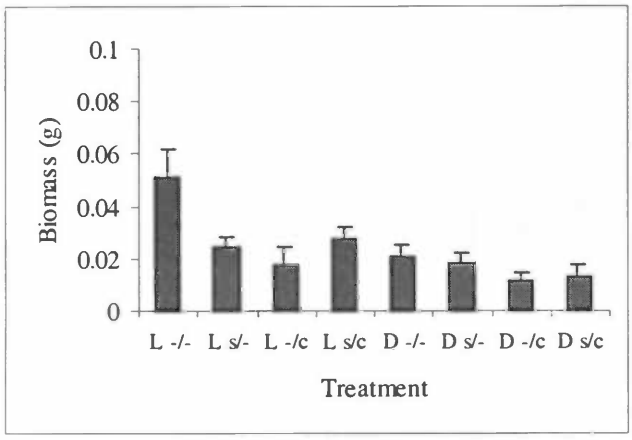



Figure a-39. The dry weight of *Sanguisorba officinalis* on clay for every treatment. Error bars are SE of means.

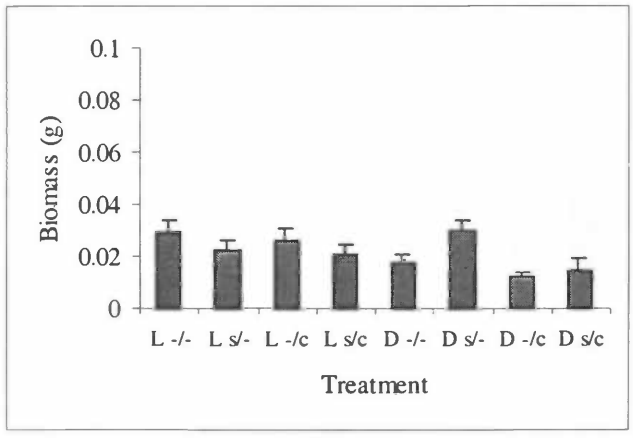



Figure a-40. The dry weight of *Sanguisorba officinalis* on peat for every treatment. Error bars are SE of means.

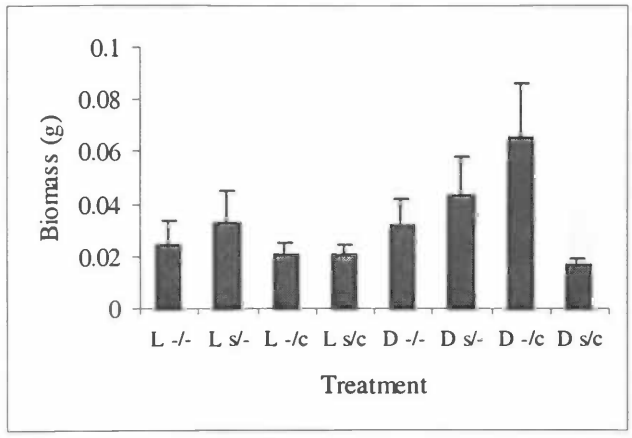



Figure a-41. The dry weight of *Sanguisorba officinalis* on sand for every treatment. Error bars are SE of means.

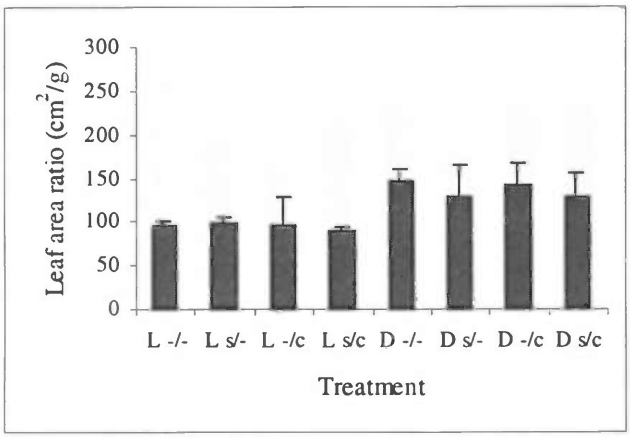



Figure a-42. The leaf area ratio of *Sanguisorba officinalis* on clay for every treatment. Error bars are SE of means.

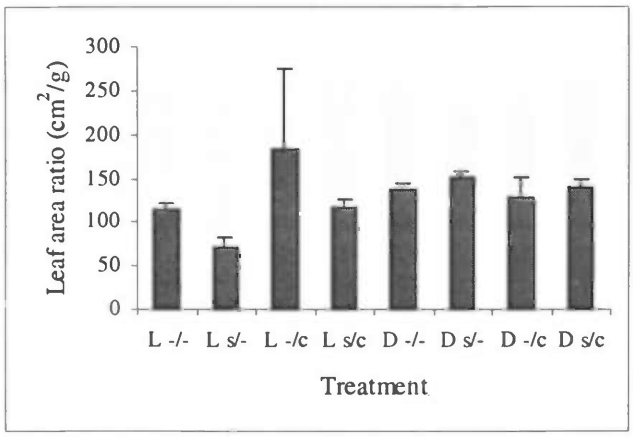



Figure a-43. The leaf area ratio of *Sanguisorba officinalis* on peat for every treatment. Error bars are SE of means.

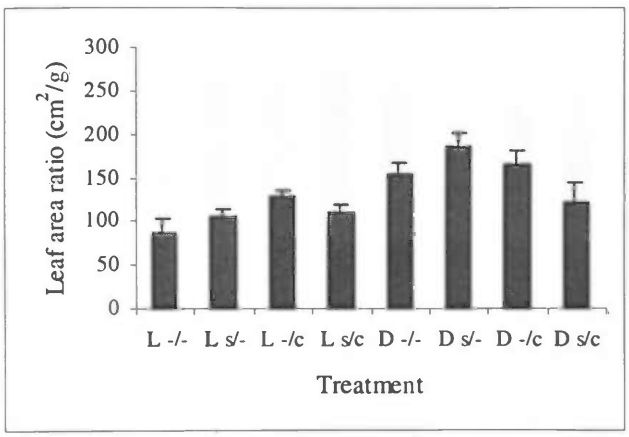



Figure a-44. The leaf area ratio of *Sanguisorba officinalis* on sand for every treatment. Error bars are SE of means.

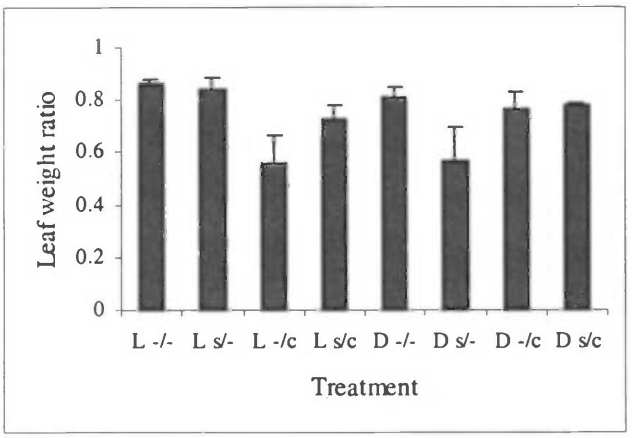



Figure a-45. The leaf weight ratio of *Sanguisorba officinalis* on clay for every treatment. Error bars are SE of means.

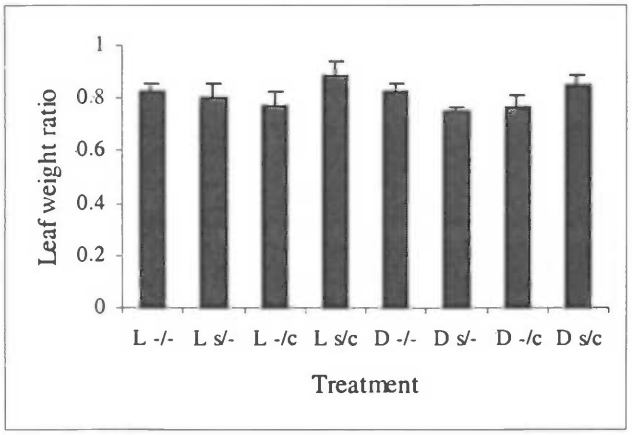



Figure a-46. The leaf weight ratio of *Sanguisorba officinalis* on peat for every treatment. Error bars are SE of means.

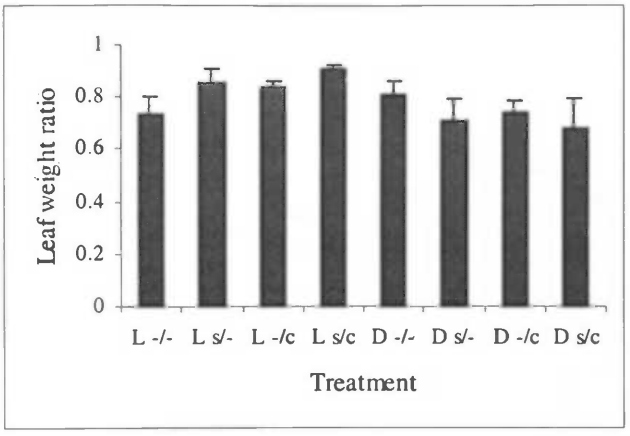



Figure a-47. The leaf weight ratio of *Sanguisorba officinalis* on sand for every treatment. Error bars are SE of means.

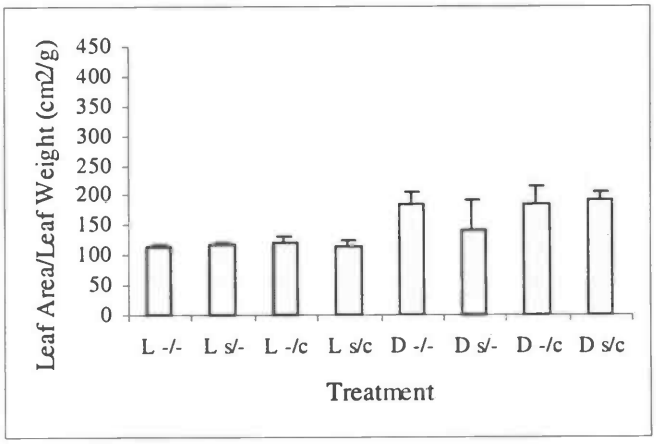



Figure a-48. leaf area/leaf weight of *Sanguisorba officinalis* on clay for every treatment. Error bars are SE of means.

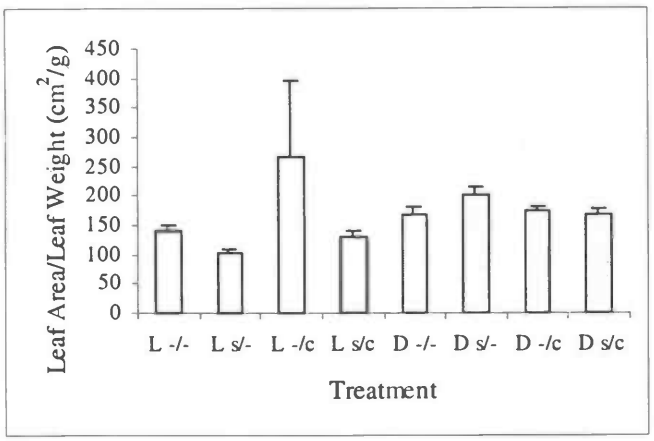



Figure a-49. leaf area/leaf weight of *Sanguisorba officinalis* on peat for every treatment. Error bars are SE of means.

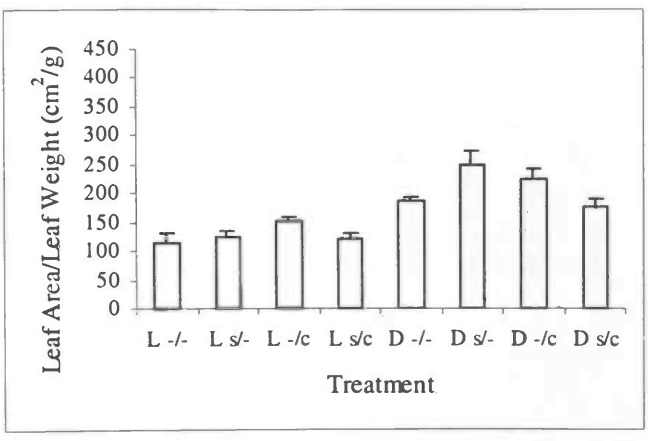



Figure a-50. The leaf area/leaf weight of *Sanguisorba officinalis* on sand for every treatment. Error bars are SE of means.

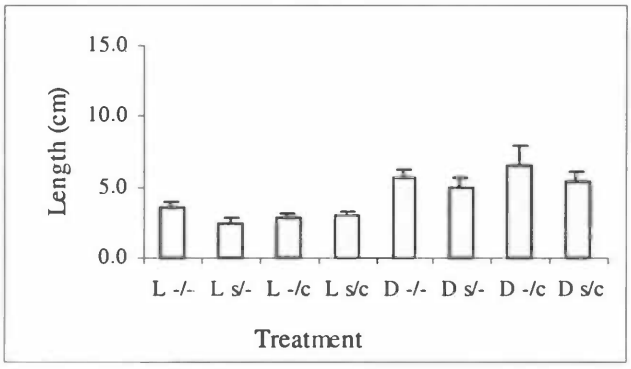



Figure a-51. The length of *Sanguisorba officinalis* on clay after 25 days for every treatment. Error bars are SE of means.

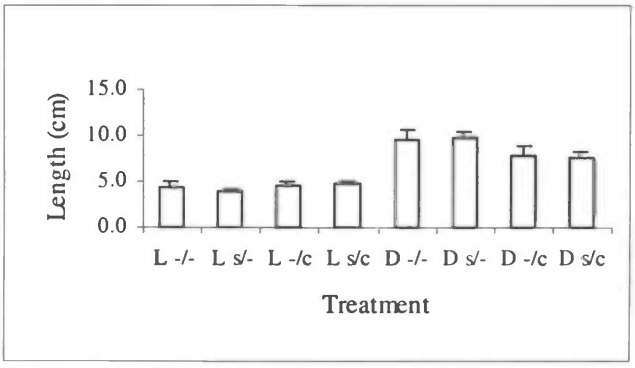



Figure a-52. The length of *Sanguisorba officinalis* on peat after 25 days for every treatment. Error bars are SE of means.

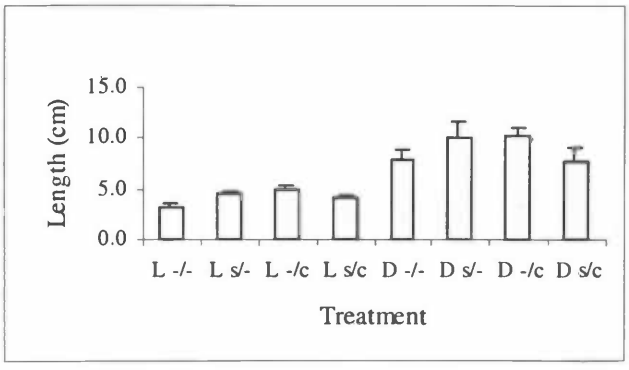



Figure a-53. The length of *Sanguisorba officinalis* on sand after 25 days for every treatment. Error bars are SE of means.

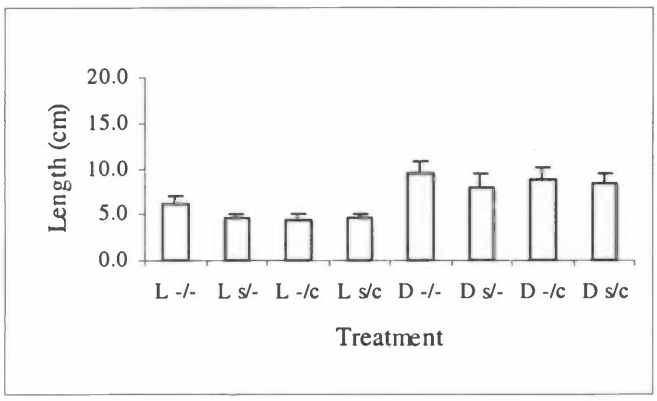



Figure a-54. The length of *Sanguisorba officinalis* on clay after 60 days for every treatment. Error bars are SE of means.

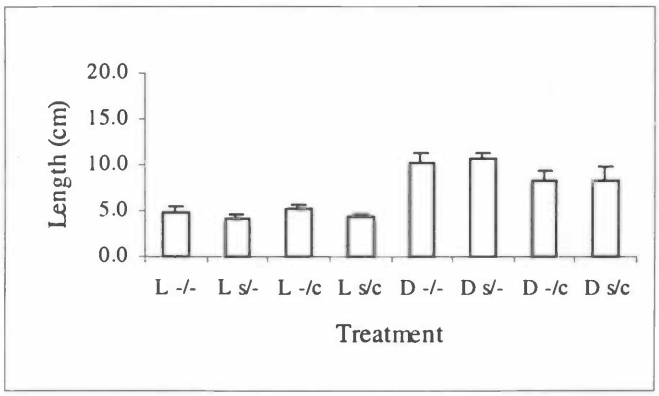



Figure a-55. The length of *Sanguisorba officinalis* on peat after 60 days for every treatment. Error bars are SE of means.

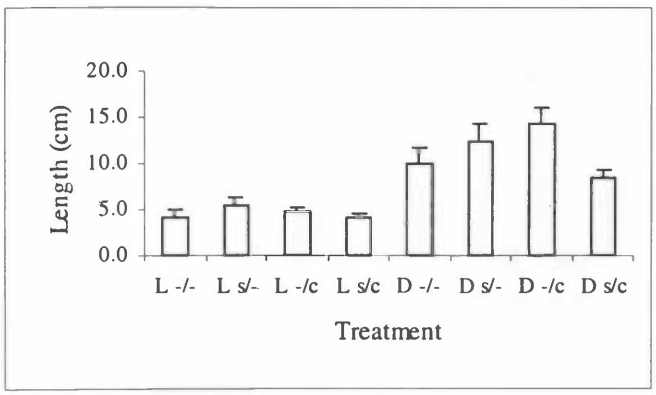



Figure a-56. The length of *Sanguisorba officinalis* on sand after 60 days for every treatment. Error bars are SE of means.

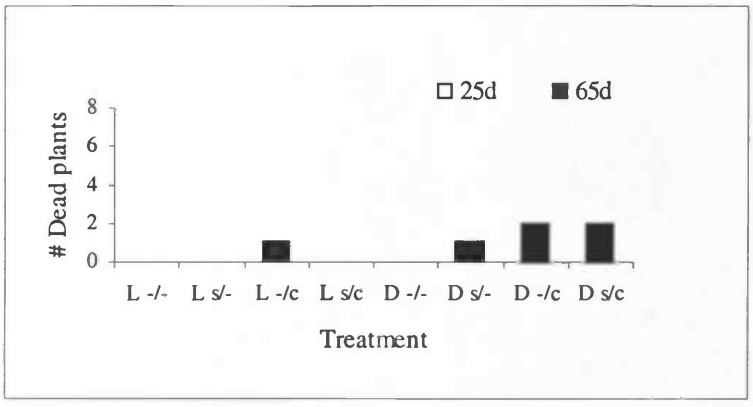



Figure a-57. The mortality (after 25 and 65 days) of *Sanguisorba officinalis* on clay for every treatment.

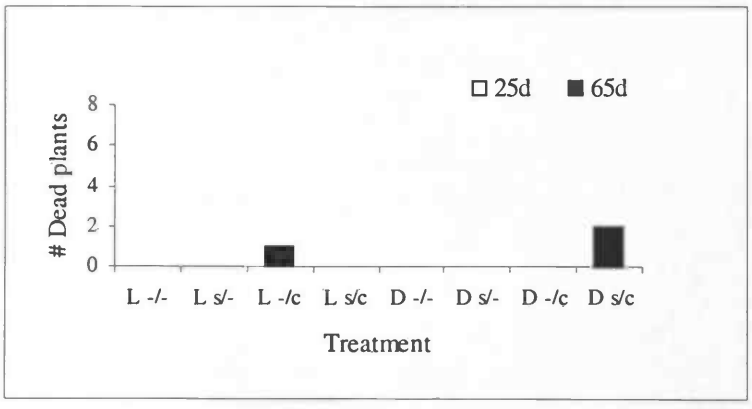



Figure a-58. The mortality (after 25 and 65 days) of *Sanguisorba officinalis* on peat for every treatment.

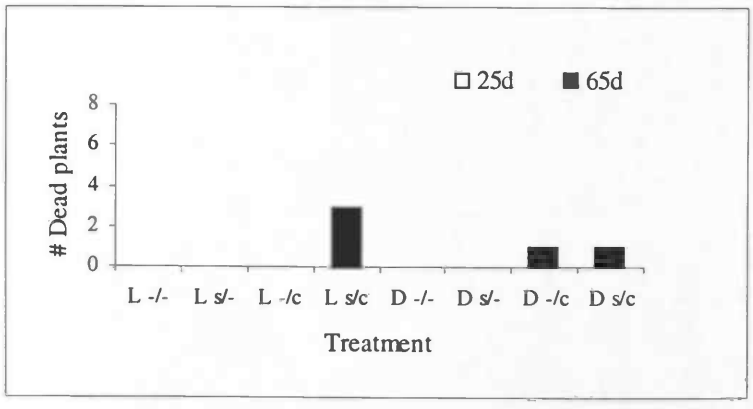



Figure a-59. The mortality (after 25 and 65 days) of *Sanguisorba officinalis* on sand for every treatment.