GERMINATION OF DUNE SLACK SPECIES ON A MICROBIAL MAT

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INTRODUCTION

Dune slacks are a very dynamic elements in coastal dunes. Vegetation development in primary and secondary dune slacks proceeds relatively rapidly.

Dune slacks harbour many Red List species. Exceptional floristic value of the young dune slacks are orchid species such as *Liparis loeselii, Dactylorhiza incarnata, Epipactis palustris, Orchis morio, Dactylorhiza maculata* and *Palanthera bifolia.* They form communities with other endangered species such as *Parnassia palustris, Schoenus nigricans, Littorella uniflora* and *Samolus valerandi* (Lammerts *et al.*, 1992).

The natural and human-included changes can lead to the decline of these communities, so the protection and conservation of pioneer vegetation on dune slacks is a main aim for nature policy and nature management in The Netherlands.

In a newly formed dune slacks the availability of nutrients is low. These poor soil conditions are maintained due to a very low rate of organic matter accumulation, very low nutrient concentration in the groundwater and a low flow rate of the groundwater (Adema, 1998). If the above conditions are stable the early succesional vegetation can exist for 30 to 80 years. It was observed that in hydrologically undisturbed dune slacks pioneer and later succesional phases can coexist for several decades (Adema, 1998).

It is not exactly known which processes are responsible for observed discontinuous behavior in dune slack ecosystem.

Adema (1998) tried to find mechanisms, which can slow down nutrient accumulation in pioneer stages and consequently prevent development of later successional stages. He suggests that the pioneer species can keep the nutrient accumulation at a low level to efficiently stabilize the pioneer stage of succession.

MICROBIAL MAT

It has been shown that microbial mats, which frequently occur on the surface of sandy soils of wet dune slacks, play an important role in development of the vegetation. Microbial mats may extend the life span of early pioneer stages during dune slack succession by

retardation the accumulation of organic matter and by inhibition the growth of later successional stages (Grootjans *et al.*, 1997, Adema, 1998).

The development of microbial mats is possible under conditions such as: not too high erosion, high availability of light and regular flooding.

The thin layer of a microbial mat on the sand surface is composed of phototrophic organisms (algae, cyanobacteria) which can produce oxygen and heterotrophic organisms (among others sulphur reducing bacteria and nitrogen reducing bacteria) which take part in the mineralization processes.

The microbial mat establishes a barrier between the atmosphere and the underground so the oxygen has a limited access to the soil. The oxygen produced by algae and cyanobacteria can penetrate in microbial mats for less than 2 mm depth in the dark to 5 – 6 mm during active photosynthesis process (Grootjans *et al.*,1997). The range of an oxic layer can not be deeper because the production of O_2 is concentrated in the top milimeters of the sediment and balanced by the intensive respiration of heterotrophic bacteria.

SULPHIDE TOXICITY

The presence of microbial mats on the top of sandy soils can cause anaerobic conditions in the lower layers. Under these conditions sulphate is reduced to sulphide by sulphur reducing bacteria according to the reaction:

$$SO_4^{2-} + 8e^- + 8H^+ = S^{2-} + 4H_2O$$

The increasing amount of sulphide in the soil environment can be toxic for plants (Lammerts *et al.*, 1992).

Some plants are able to avoid toxic effects by the oxidation of sulphide in the rizosphere (Havill *et al.*, 1985). Some of plants which colonize bare areas during pioneer successional stages are able to leak oxygen from their roots (**R**adial **O**xygen **L**oss – **ROL**) and creation a non – reducing zone in the rizosphere. (Adema, 1997)

Samolus valerandi, *Schoenus nigricans* and *Littorella uniflora*, which are dominating in the first phase of primary succesion, have special morpho-physiological adaptations to leak oxygen to the soil in order to protect themselves from unfavorable conditions.

Later successional species, such as *Calamagrostis epigejos* and *Carex nigra*, are not capable of ROL and they can tolerate considerable less sulphide in the sandy soil compared to pioneer species (*C. epigejos* can tolerate 30 – 50 μM sulphide) (Grootjans *et al.*, 1997).

Adema has shown that in mesocosms sulphide can occur at least at a depth of 2 cm. Therefore seedlings of dune species may grow in an environment in which sulphide is present. This sulphide can influence germination of seeds or can kill seedlings after germination.

ERLIER RESEARCHES

Grootjans *et al.* (1997) found that microbial mats can influence the survival of seedlings from three plant species representanting different successional stages in dune slack development. In this experiment seedlings of *Samolus valerandi, Calamagrostis epigejos* and *Juncus alpinoarticulatus* were placed on the top of the microbial mat under different conditions. 26-weeks of observation showed that *S. valerandi* was not affected negatively by a well-developed microbial mat. The decreasing percentage of the survival of seedlings was observed when the water level was 1.5 cm below the soil surface and under 'moist' condition. *C. epigejos* and *J. alpinoarticulatus* showed a poor growth in already established microbial mat.

Bengtsson (1999) compared the germination process of six dune slack species, three pioneers and three later. These species were tested for the impact of the different treatments: mat – no mat and low S – high S. The mat – no mat treatments mean that plants were growing with or without the microbial mat on the surface of the canisters. The low S – high S treatments mean that some canisters were supplied with low sulphide concentrations, and other with high sulphide concentrations in the artificial groundwater. Bengtsson found significant differences in the numbers of seedlings of *S. valerandi* and *C. epigejos* between the mat – no mat treatment. Both species germinated better in the no mat treatment than the mat treatment.

Bengtsson (1999) also found that large differences within the mat treatments with low sulphide concentration: the numbers of seedlings in three vessels were higher then in the

fourth vessel. These observation lead to the hypothesis that there can be large differences between mats.

RESEARCH QUESTIONS

If indeed differences between microbial mats have an effect, this also may have influence on the vegetation development in early succesional stages.

Moreover we also want to know if we can measure differences between microbial mat activity by measuring the oxygen production of these mats.

This leads to the following research questions:

- **Are there differences in the number of seeds that germinate on a microbial mat from different dune slacks and without a microbial mat?**
- **Can we determine the activity of a microbial mat by measuring the oxygen production?**

MATERIAL AND METHODS

GERMINATION AND SURVIVAL PROCESSES

The germination of seeds and the survival of seedlings were tested in six mesocosms (60-40-20) cm, constructed to imitate physical properties of a young dune slack ecosystem (Fig.1.). Every mesocosm was composed of three main elements: a bottle of artificial groundwater, a container and a pomp.

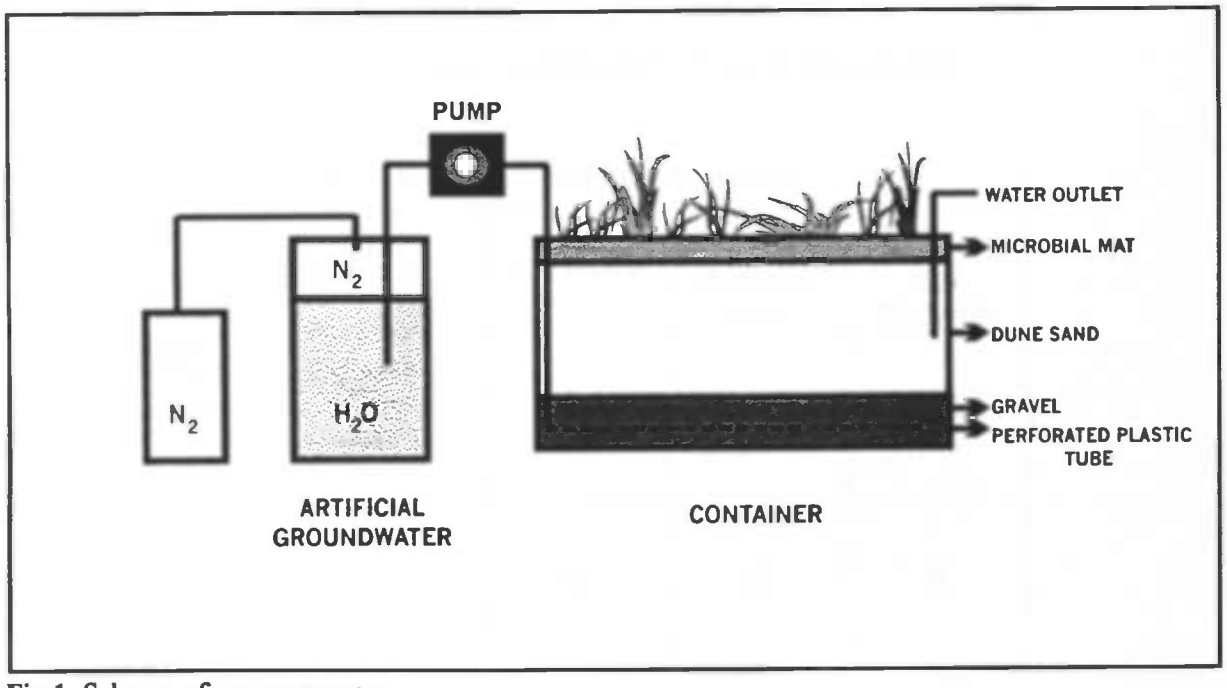

Fig.1. Scheme of one mesocosm.

Artificial groundwater had the same composition as the groundwater from Het Kapenglop on the island of Texel (the Netherlands) (Appendix 1.). The artificial groundwater was given at the bottom of each container through perforated plastic tube to simulate seepage of groundwater. The water outlet, which is placed on the soil surface level, kept the water table stable. The water was kept anaerobic with nitrogen gas at the top of the bottle of artificial grounwater.

At the bottom of each container a small layer of gravel was present to avoid unequal distributions of water.

The plastic containers were filled with calcareous dune sand originated from the beach of Texel (the Netherlands).

The soil surface of four of the containers (Fig.1.) was covered by a microbial mat; two containers with a mat from 'De Buiten Muy' and two with a mat from 'Het Kapenvlak'. To investigate the general effect of a microbial mat on the germination and the survival processes the last two containers were kept without a microbial mat.

Each of the six containers was divided into 4 units and each of these units into 8 subunits. This was done to get 4 replicas of 8 species in each container (Table 1.).
Seeds from all each species were sown in five rows , 10 seeds in each row.

Table 1. The number of repeats in the different treatments.

SPECIES	NO MAT	WITH MAT	
		De Buiten Muy	Het Kapenvlak
Pioneer species: • *Samolus valerandi* • *Schoenus nigricans* • *Agrostis stolonifera* • *Parnassia palustris*	8	8	8
Later species: • *Carex nigra* • *Calamagrostis epigejos* • *Phragmites australis* • *Pedicularis palustris*	8	8	8

Eight dune slack species were tested for the impact of three different treatments: no mat, a microbial mat from 'De Buiten Muy' and a microbial mat from 'Het Kapenvlak' (Table 1).The used plants represent the different successional stages of dune slack vegetation. *Samolus valerandi, Schoenus nigricans, Agrostis stolonifera, Parnassia palustris* were selected from pioneer species and *Calamagrostis epigejos, Carex nigra, Phragmites australis* and *Pedicularis palustris* are representative for the later stages of succession (Table 1.).

To determine the germination ability of the used seeds, four times 50 seeds of each species were placed in petridishes in a climate chamber and kept under moist

conditions. The percentage of germinated and survived seeds in the containers was corrected on the base the number of germinated seeds in the petridishes.

The containers as well as the petridishes were placed in a climate chamber with a 12/12 hour day night cycle. The temperature was 25°C during the day and 15°C during the night.

The seedlings were counted twice a month and marked with a plastic stick, every time in a different color.

The unwanted species, which were growing in the containers during the whole experiment, were clipped at the ground surface (to prevent removal of seeds by pulling out the roots).

THE ACTIVITY OF A MICROBIAL MAT

The activity of the microbial mat was determined by measuring the oxygen production.

To measure the oxygen production we used a Clark type oxygen electrode (Strathkelvin 1302 Microcathode Oxygen Electrode) in a headspace (16 cm height, 7 cm diameter) placed on the surface of the microbial mat (Appendix 13.).

Since the Clark type oxygen electrode measures the partical pressure of oxygen it is very sensitive for pressure changes. To prevent pressure differences, while we measured, we placed a capillary tube in the headspace (Appendix 13.).

On theoretical grounds we assumed a linear increase of the oxygen concentration. We measured the oxygen concentration each 2 seconds for 5 minutes, on each site with 5 replicas. In this time we do not expect significiant differences in the oxygen production rate so the increase of oxygen concentration in time will be linear (B) (Fig.2.).

However if we measure the oxygen concentration in a headspace this relation is not longer linear. We rather assume a saturation curve (A) because of leakage (capillary tube) and inhibition of oxygen production (Fig.2.).

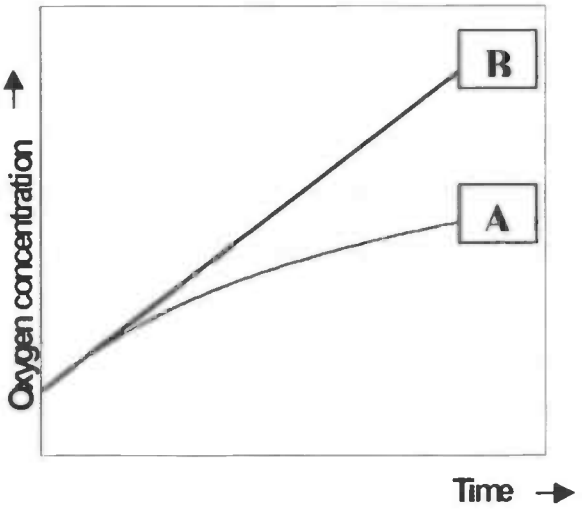

Fig.3. The measured (A) and real (B) oxygen production of a microbial mat.

We can describe this curve (A) by the following equation:

$$\%O_2 = \%O_{2(0)} + M\frac{t}{t+h} \qquad (1)$$

where $\%O_2$ – oxygen conentration on time t;
$\%O_{2(0)}$ – oxygen concentration on time 0;
M – maximum- increase of $\%O_2$;
t – time;
h – half saturatione constant;

To find the oxygen production rate we have to calculate the speed at time zero (t=0). We can do this by differentiation of equation (1) this gives:

$$\frac{d\%O_2}{dt} = \frac{M}{t+h} + \frac{Mt}{(t+h)^2} \qquad (2)$$

For t=0 it will be: $$\frac{d\%O_2}{dt_{(0)}} = \frac{M}{h} \quad (3)$$

The microbial mats are influenced by the temperature and light availability.The amount of light and temperature were also collected each 2 seconds for 5 minutes, on each site with 5 repicas. The light was measured with a photocell and the temperature with a termistor, both placed in the headspace.

To determine correlations between the oxygen production rate and the temperature and also between the oxygen production rate and the availability of light a Spearman's rho Test was used (Appendix 10.).

The Clark type oxygen electrode, the light sensor and the termistor were calibrated once in the end of the experiment (Appendix 12.).

RESULTS

The percentage of seeds that had germinated in the different treatments are presented on the Figure 4 (see in Appendix 3-5).

The seeds from *Pedicularis palustris* and *Parnassia palustris* did not germinate either in the petridishes (Appendix 2.) nor in the containers (Appendix 3-5).

Significant differences in the percentage of germinated seeds were found using the Kruskal – Wallis Test (Appendix 9.). Significance of differences was tested on the two levels of $p<0.01$ and $p<0.05$.

Significant differences in the percentage of germinated seeds from *Agrostis stolonifera* were found between the mat from 'De Buiten Muy' and the mat from 'Het Kapenvlak'. This early successional species germinated considerably better on the microbial mat from 'Het Kapenvlak' than from 'De Buiten Muy' ($p<0.05$ after 26 and 46 days) ($p<0.01$ after 60 and 75 days) (Appendix 9.). In addition the number of seedlings from *A.stolonifera* in the containers with the microbial mat from 'Het Kapenvlak' was almost two times higher than the number of seeds that germinated in the petridishes.

For *S.valerandi* no differences were found between three treatments (Appendix 9.). Differences between the no mat treatment and containers with the microbial mat appeared after 60 days of experiment.

The seeds from *Schoenus nigricans* germinated better without the microbial mat than in the treatments with the microbial mat. During the first 45 days of experiment the number of seedlings in the containers with mat from 'Het Kapenvlak' was higher than in the containers with mat from 'De Buiten Muy'. After this period germination improved on mat collected from 'De Buiten Muy' (Appendix 9.).

Significant differences between two mat tratments could be noticed for *Carex nigra* after 60 days. On the microbial mat from 'Het Kapenvlak' the first seedling of *C.nigra* could be counted only after 60 days while seedlings in the other treatments were presented after 26 days.

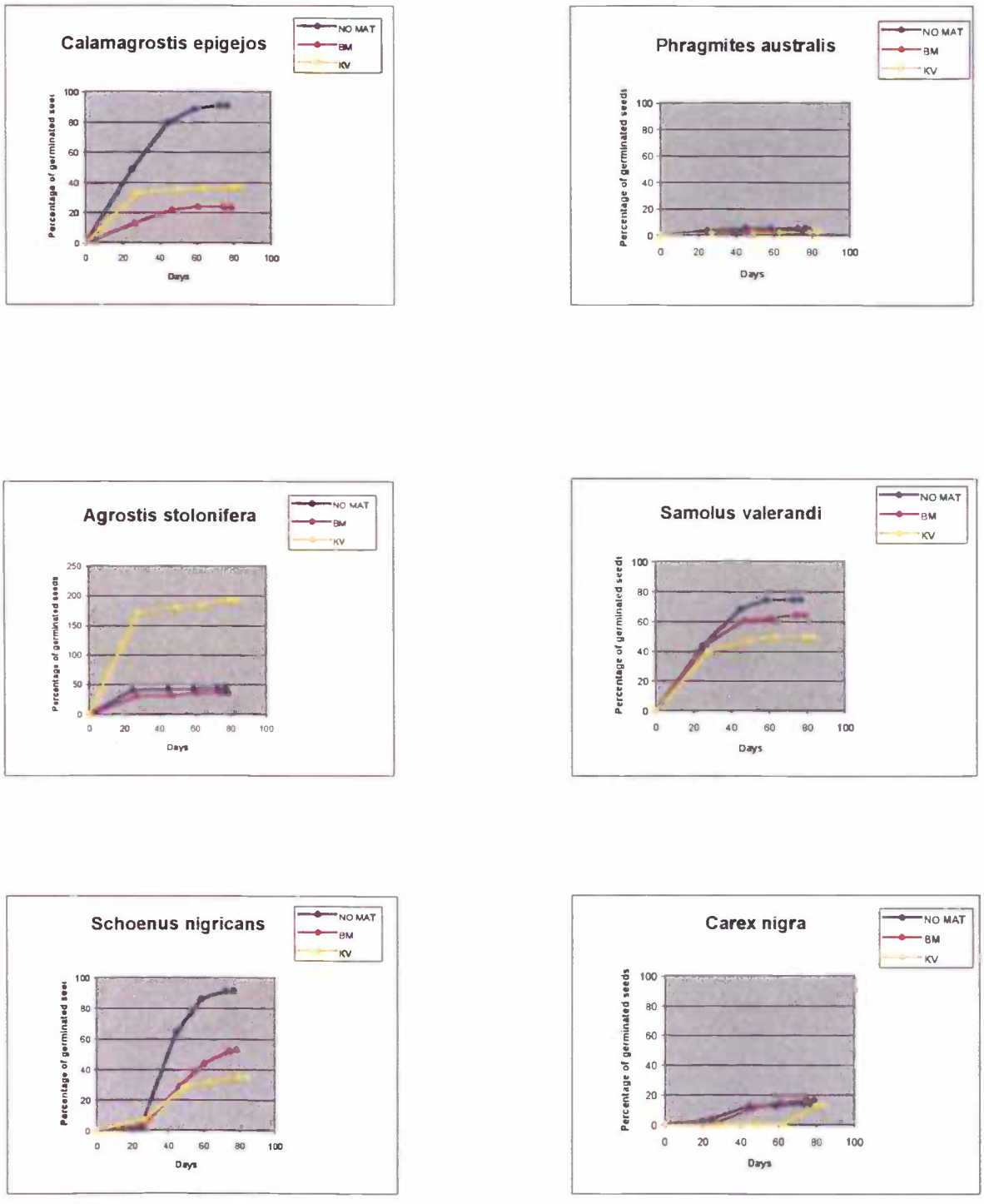

Fig.4. The influence of three different treatments (no mat, mat from 'De Buiten Muy' and mat from 'Het Kapenvlak') on the percentage of germinated seeds of six dune slack species.

In the case of *Calamagrostis epigejos* the percentage of germinated seeds in the no mat treatment reached about 90%. The number of seedlings on the end of this experiment was the lowest for the containers with the microbial mat from 'De Buiten Muy' and with the microbial mat from 'Het Kapenvlak'. Significant differences were found between the percentage of germinated seeds in the no mat treatment and the mat from 'De Buiten Muy'. The number of seedlings in the containers with the microbial mat from 'Het Kapenvlak' were much higher than in the containers with the microbial mat from 'De Buiten Muy' after 45 days.

Phragmites australis, from which almost all seeds germinated in the petridishes has only a few seedlings in the containers (Appendix 2-5). The statistic test revealed the significant differences in the growth of this species on the microbial mat from 'Het Kapenvlak' and without the microbial mat. The seeds from *P.australis* germinated considerably better on the microbial mat from 'Het Kapenvlak'.

Almost all germinated seeds survived until the end of the experiment. Only a few seedlings of *A.stolonifera, S.valerandi* and *S.nigricans* died (on the microbial mat from 'De Buiten Muy' and 'Het Kapenvlak'). The mortality rate of seedlings was highest in the no mat treatment.

OXYGEN MEASUREMENT

Figure 5. shows a typical oxygen measurement of a microbial mat under the headspace.

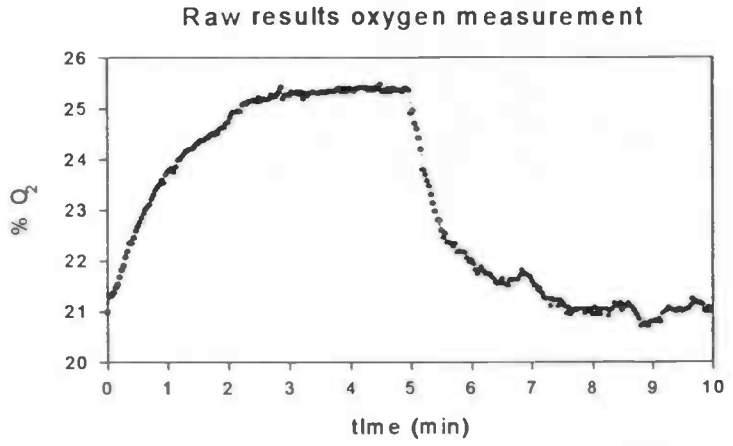

Fig.5. The typical oxygen production measurement.

We measured the oxygen production during the first five minutes. The next five minutes were used to decrease the oxygen concentration in the Clark electrode to 21%. (Fig.5.).

We calculated the oxygen production rate from the oxygen measurements. The calculated oxygen production rate was transformed to a logaritmic scale to get homogeneity of variances. A oneway Anova test showed significant ($p<0.001$) differences between the sites. A Student–Newman–Keuls multiple comparison test was used to test differences between the sites. This test separated measurements in five groups for eight different sites (as is shown on Fig.6.).

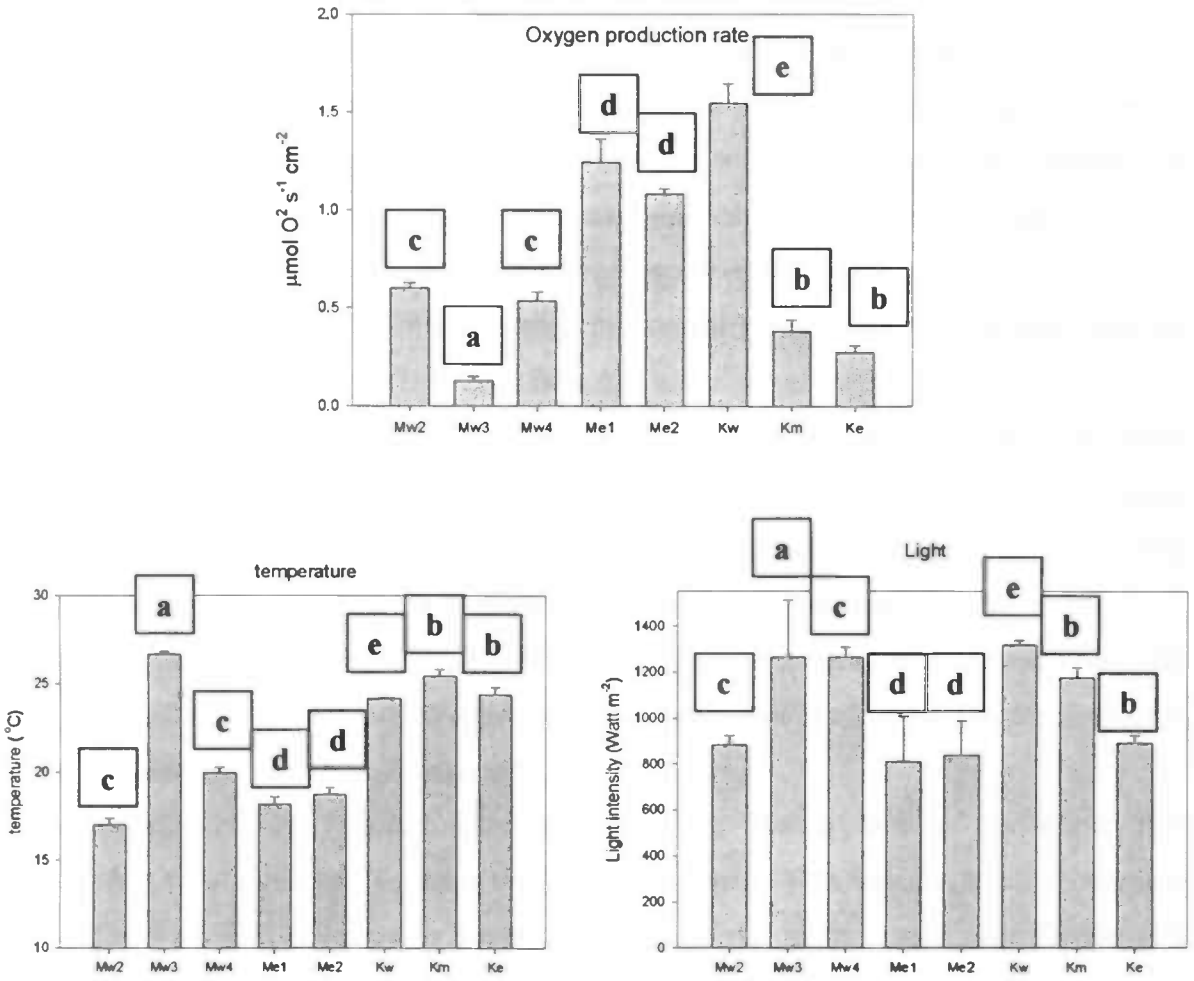

Fig.6. The temperature, light and oxygen production rate measurements in each site of measurement.

The rate of oxygen production can be limited by the amount of light or the temperature.

The Spearman's rho statistical test assessed a negative correlation between temperature and oxygen production rate. There is no correlation between the oxygen production rate and the availability of light (Appendix 10.)

DISCUSSION

GENERAL EFFECT OF THE PRESENCE OF A MICROBIAL MAT

The presence of a microbial mat on the soil surface limited the germination of *S.nigricans, C.nigra, C.epigejos* and *P.australis*. It could be caused by the presence of sulphide or the physical properties of a microbial mat. The microbial mat can be a physical barrier between the atmosphere and the mineral soil.

A positive influence of a microbial mat on the germination process was observed in the case of *A.stolonifera*. The seeds from this species preferred the microbial mat from 'Het Kapenvlak' even better than the optimal conditions in the petridishes. It seems likely that *A.stolonifera* is stimulated by this microbial mat. There is may be an other growing factor in the microbial mat, which is also needed for the germination process than only sufficient temperature, moist and light availability in the petridishes.

S valerandi shows no differences between the containers with the microbial mat and containers without the microbial mat. The number of seedlings from this species is more or less similar in both treatments.

Grootjans *et al* (1997) found very similar results. His experiment has shown that *S.valerandi* survived better under very wet conditions. This species was not affected negatively by well-developed microbial and algal mats. In this experiment *S.valerandi* grew poor when the water table was lowered by only 1.5 cm.

DIFFERENCES BETWEEN A MICROBIAL MAT OF 'HET KAPENVLAK' AND 'DE BUITEN MUY'

Significant differences between microbial mats could be noticed in the case of *A.stolonifera*. The germination of seeds from this species was stimulated by microbial mat from 'Het Kapenvlak' (more than by optimal conditions in the petridishes) and restricted by the microbial mat from 'De Buiten Muy'. The two containers with the microbial mat from 'Het Kapenvlak' show different numbers of seedlings. The numbers of germinated seeds from *A.stolonifera, S.valerandi, C.epigejos* and *P.australis* were

much higher in the first container than in the second one. It seems likely that the first container was wetter than the second one.

The presence of the microbial mat from 'De Buiten Muy' had no influence on the survival of seedlings of *C.nigra*. This in contrast to the microbial mat from 'Het Kapenvlak'. This treatment inhibited the germination of *C.nigra*. The lower germination rate could be caused by the water conditions as mentioned before.

The kind of interaction (positive or negative) between the microbial mat and plants that grew on it may influence in the establishment of plant species in dune slacks.

Differences in the percentage of seeds that germinated on the microbial mat indicate that these mats have an effect on the development of particular dune slack species and therefore on the succession.

Problems with one pump could have an influence on the results. This pump was not performing well during the experiment. The unequal imput of artificial groundwater can explain unequal growth of particular species in the containers fet by this pump.

Seeds of *Parnassia palustris* and *Pedicularis palustris* did not germinate either in the containers nor in the petridishes. The reason of this strange behavior could be the loss of viability of these old seeds.

CONCLUSION

This experiment should significant differences between the number of seeds that germinated on a microbial mat from 'Het Kapenvlak' and from 'De Buiten Muy'. The microbial mats play an important role in the germination process. This study revealed that the development of dune slack species could be stimulated or inhibited by microbial mats.

OXYGEN MEASUREMENTS

During the five oxygen production measurements in the same sites only little variation existed. Considerably variation existed between the sites in the oxygen production rate which could be caused by differentiation of microbial mats.

The mat's activity is depended on environmental factors as water table, nutrient availability, light and temperature.

CORRELATION BETWEEN THE TEMPERATURE AND THE OXYGEN PRODUCTION RATE

This experiment showed a negative correlation between the temperature and the oxygen production rate.

Brock *et al* (1997) explained positive and negative effect of temperature on the microbial growth as follows: the increase of temperature stimulates chemical and enzymatic reactions in the cell and growth becomes faster. The increment of biomass could be responsible for increased photosynthesis and oxygen production rate. If the temperature is very low some cellular components (nucleic acids, proteins) can be damaged. Therefore each microorganism has an optimal temperature in which growth is best (for cyanobacteria about 40-60°C).

No differences were found between light availability and oxygen production rate. It was very sunny during the oxygen production measurements and the range of light intensity was between 800 and 1300 Watt m^{-2}. Seliger *et al* (1965) found that the saturation of photosynthesis occures when light intensity is above 200 Watt m^{-2}.

We observed in the field that if the headspace with the Clark electrode is not in a vertical position, the electrode give a less stable signal. So to prevent large deviations between the measurements it is important to keep the headspace upright.

CONCLUSION

"A good method is the method which can be repeated with the same good results"

In this experiment the repeated measurements of oxygen production rate (activity of microbial mat) were very similar in the same site.

We can distinguish difference in microbial mat activity in different sites by measuring oxygen production rate.

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Appendix 1.

Composition of artificial ground water

The composition of artificial ground water was based on the ground composition of the ground water from dune slacks on Schiermonnikoog (the Netherlands) (Erwin Adema, pers. comm.):

Cations	mmol/l	mg/l
Ca^{2+}	2.63	105.5
Na^{+}	3.96	91.17
K^{+}	0.10	3.91
Mg^{2+}	0.33	7.90
NH_4^{+}	0.60	10.80
$Fe(II)^{2+}$	0.01	0.38
Anions		
SO_4^{2-}	1.31	126.31
Cl^{-}	3.98	141.18
$H_2PO_4^{-}$	0.00	0.05
Gas		
CO_2	1.50	65.93

Appendix 2. THE NUMBER OF GERMINATED SEEDS IN THE PETRIDISHES

SPECIES	AFTER 7 DAYS					AFTER 13 DAYS					AFTER 17 DAYS					AFTER 21 DAYS					AFTER 27 DAYS					AFTER 32DAYS					AFTER 39 DAYS					TOTAL NUMBER OF GERMINATED SEEDS
	Repeats				Average	Repeats				Average	Repeats				Average	Repeats				Average	Repeats				Average	Repeats				Average	Repeats				Average	
Agrostis stolonifera	2	6	7	5	5	1	2	1	2	1.5	1	0	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.25
Samolus valerandi	0	0	0	0	0	22	31	20	27	25	6	4	5	4	4.5	2	2	1	1	1.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31
Schoenus nigricans	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0.75	4	5	5	2	4.75	5	3	8	11	6.75	6	12	10	9	9.25	10	3	10	8	7.75	28.5
Carex nigra	0	0	0	0	0	0	2	2	3	1.75	1	2	2	3	2	1	2	1	1	1.25	9	3	2	1	3.75	0	0	1	2	0.75	0	0	1	0	0.25	9.75
Calamagrostis epigejos	0	0	0	0	0	2	9	7	4	5.5	6	0	1	0	1.75	1	1	5	1	2	0	2	0	1	0.75	0	2	2	0	1	0	0	1	0	0.25	11.25
Phragmites australis	50	41	45	44	45	0	6	2	2	2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47.5
Parnassia palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pedicularis palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3. NUMBER OF SEEDS THAT GERMINATED IN THE NO MAT TREATMENT.

NO MAT

SPECIES	7 April - 2 May								Average	2 May - 22 May								Average	22 May - 5 June								Average	5 June -19 June								Average	19June-23June								Average	Total number of germinated seeds	Percentage of germinated seeds
	After 25 days									After 45 days									After 59 days									After 73 days									After 77 days										
Agrostis stolonifera	5	3	5	5	1	1	0	4	3	0	0	0	1	0	0	0	0	0.125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.125	41.3
SE*									± 0.3									± 0.05									± 0									± 0									± 0		
Samolus valerandi	14	11	12	16	10	16	12	17	13.5	4	4	8	5	12	8	8	13	7.75	2	1	2	1	3	5	0	1	1.875	0	0	0	0	1	0	0	0	0.125	0	0	0	0	0	0	0	0	0	23.25	75
SE*									± 0.37									± 0.49									± 0.22									± 0.05									± 0		
Schoenus nigricans	4	0	0	0	0	0	0	1	0.75	12	9	12	19	19	24	24	23	17.75	9	5	11	8	3	4	4	5	6.125	4	3	0	1	1	1	1	1	1.5	0	0	0	0	0	0	0	0	0	26.125	91.67
SE*									± 0.2									± 0.86									± 0.41									± 0.19									± 0		
Carex nigra	0	0	1	0	0	1	1	0	0.375	1	0	0	1	0	1	2	2	0.875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.125	0	0	0	0	0	0	0	0	0	1.375	14.11
SE*									± 0.07									± 0.12									± 0									± 0.05									± 0		
Calamagrostis epigejos	9	4	6	4	3	6	4	7	5.5	0	3	2	4	6	1	7	5	3.5	0	1	0	0	1	2	3	1	1	0	1	1	0	0	0	0	0	0.25	0	0	0	0	0	0	0	0	0	10.25	91.11
SE*									± 0.29									± 0.35									± 0.15									± 0.07									± 0		
Phragmites australis	2	0	3	1	1	3	1	3	1.75	0	1	0	1	1	0	2	1	0.75	0	0	0	0	0	1	0	0	0.125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.625	5.53
SE*									± 0.17									± 0.1									± 0.05									± 0									± 0		
Parnassia palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SE*									± 0									± 0									± 0									± 0									± 0		
Pedicularis palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SE*									± 0									± 0									± 0									± 0									± 0		

* Standard Error

Appendix 4. NUMBER OF SEEDS THAT GERMINATED ON A MICROBIAL MAT FROM 'DE BUITEN MUY'

DE BUITEN MUY

SPECIES	7 April - 2 May									2 May - 22 May									22 May - 5 June									5June -19 June									19June -23June									Total number of	Percentage of
	After 27 days								Average	After 47 days								Average	After 61 days								Average	After 75 days								Average	After 79 days								Average	germinated seeds	germinated seeds
Agrostis stolonifera	5	3	6	0	0	4	0	1	2.375	1	0	0	0	0	0	0	0	0.125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.75	36.67
SE*									± 0.35									± 0.29									± 0									± 0									± 0		
Samolus valerandi	8	2	9	8	20	15	25	24	13.875	8	1	3	7	8	5	3	5	5	0	0	0	0	2	2	1	0	0.625	1	0	0	1	1	1	0	0	0.5	0	0	0	0	0	0	0	0	0	15.125	64.52
SE*									± 1.2									± 1.05									± 1.02									± 0.53									± 0		
Schoenus nigricans	4	2	0	2	0	0	0	0	1	9	5	24	11	2	7	1	0	7.375	7	6	8	8	0	4	1	0	4.25	3	7	4	4	0	1	0	0	2.375	0	0	0	0	1	0	0	0	0.125	2.5	53.07
SE*									± 0.22									± 0.88									± 0.75									± 0.37									± 0.05		
Carex nigra	0	0	0	0	0	0	0	1	0.125	2	1	3	2	0	0	0	0	1	0	0	0	1	1	0	0	1	0.375	0	1	0	0	0	0	0	0	0.125	0	0	0	0	0	0	0	0	0	1.625	16.67
SE*									± 0.05									± 0.13									± 0.12									± 0.05									± 0		
Calamagrostis epigejos	4	3	0	4	1	0	0	0	1.5	1	3	0	4	0	0	0	0	1	1	0	0	0	1	0	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.75	24.44
SE*									± 0.26									± 0.24									± 0.2									± 0									± 0		
Phragmites australis	0	1	1	2	3	0	0	1	1	0	2	1	0	0	1	0	0	0.5	1	1	0	1	0	0	0	0	0.375	0	1	0	0	0	0	0	0	0.125	0	0	0	0	0	0	0	0	0	2	4.21
SE*									± 0.15									± 0.13									± 0.11									± 0.05									± 0		
Parnassia palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SE*									± 0									± 0									± 0									± 0									± 0		
Pedicularis palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SE*									± 0									± 0									± 0									± 0									± 0		

* Standard Error

Appendix 5. NUMBER OF SEEDS THAT GERMINATED ON A MICROBIAL MAT FROM 'HET KAPENVLAK'

HET KAPENVLAK

SPECIES	7 April - 2 May								Average	2 May - 22 May								Average	22 May - 5 June								Average	5 June -19 June								Average	19June-23June								Average	Total number of germinated seeds	Percentage of germinated seeds
	After 27 days									After 47 days									After 61 days									After 77 days									After 81 days										
Agrostis stolonifera	2	3	6	5	27	19	25	15	12.75	1	0	0	2	0	1	0	1	0.625	3	0	0	0	0	0	0	0	0.375	3	0	0	0	3	0	0	0	0.75	0	0	0	0	0	0	0	0	0	14.5	193.33
SE*									± 1.4									± 0.1									± 0.1									± 0.2									± 0		
Samolus valerandi	6	10	6	6	26	26	4	13	12.125	4	2	3	0	3	4	2	1	2.375	1	1	2	0	1	0	0	1	0.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.25	49.19
SE*									± 1.3									± 1.1									± 1									± 0									± 0		
Schoenus nigricans	0	1	3	0	7	5	0	0	2	6	6	8	14	4	8	3	0	6.125	3	3	0	1	0	0	0	0	0.875	0	0	2	2	0	1	0	1	0.75	1	0	0	0	0	1	0	0	0.25	10	35.09
SE*									± 0.39									± 0.6									± 0.5									± 0.13									± 0.3		
Carex nigra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0.125	0	0	0	0	0	0	0	0	0	0.125	12.82
SE*									± 0									± 0									± 0									± 0.05									± 0		
Calamagrostis epigejos	0	2	5	3	4	9	3	3	3.625	1	0	0	1	0	0	0	0	0.25	0	1	0	0	0	0	0	0	0.125	0	0	0	1	0	0	0	0	0.125	1	0	0	0	0	0	0	0	0.125	4.25	37.78
SE*									± 0.4									± 0.3									± 0.3									± 0.05									± 0.05		
Phragmites australis	0	0	0	0	1	4	0	1	0.75	0	0	0	0	1	1	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.11
SE*									± 0.2									± 0.1									± 0									± 0									± 0		
Parnassia palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SE*									± 0									± 0									± 0									± 0									± 0		
Pedicularis palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SE*									± 0									± 0									± 0									± 0									± 0		

* Standard Error

Appendix 6. NUMBER OF SEEDLINGS THAT DID NOT SURVIVED IN THE NO MAT TREATMENT

NO MAT

SPECIES	7 April - 2 May After 25 days	Average	2 May - 22 May After 45 days	Average	22 May - 5 June After 59 days	Average	5 June -19 June After 73 days	Average	19June-23June After 77 days	Average	Total number of seedlings that did not survive	Percentage of seedlings that did not survive
Agrostis stolonifera	0 0 0 0 0 0 0 0	0	0 0 0 1 0 0 0 0	0.125	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0.125	1.72
Samolus valerandi	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 4	0.5	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0.5	1.61
Schoenus nigricans	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 1	0.125	0 0 0 0 0 0 0 0	0	0.125	0.44
Carex nigra	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Calamagrostis epigejos	0 0 0 0 0 0 0 0	0	0 0 0 0 0 2 0 0	0.25	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0.25	2.22
Phragmites australis	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 1	0.125	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0.125	0.26
Parnassia palustris	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Pedicularis palustris	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0

Appendix 7. NUMBER OF SEEDLINGS THAT DID NOT SURVIVE ON A MICROBIAL MAT FROM 'DE BUITEN MUY'

DE BUITEN MUY

SPECIES	7 April - 2 May After 25 days	Average	2 May - 22 May After 45 days	Average	22 May - 5 June After 59 days	Average	5 June -19 June After 73 days	Average	19June-23June After 77 days	Average	Total number of seedlings that did not survive	Percentage of seedlings that did not survive
Agrostis stolonifera	0 0 0 0 0 0 0 0	0	0 1 0 0 0 0 0 0	0.125	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0.125	1.72
Samolus valerandi	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 1	0.125	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0.125	0.4
Schoenus nigricans	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 1 0 0	0.125	0 0 0 0 0 0 0 0	0	0.125	0.44
Carex nigra	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Calamagrostis epigejos	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Phragmites australis	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Parnassia palustris	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Pedicularis palustris	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0

Appendix 8. NUMBER OF SEEDLINGS THAT DID NOT SURVIVE ON THE MICROBIAL MAT FROM 'HET KAPENVLAK'

HET KAPENVLAK

SPECIES	7 April - 2 May After 25 days	Average	2 May - 22 May After 45 days	Average	22 May - 5 June After 59 days	Average	5 June -19 June After 73 days	Average	19June-23June After 77 days	Average	Total number of seedlings that did not survive	Percentage of seedlings that did not survive
Agrostis stolonifera	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 1	0.125	0 0 0 0 0 0 0 0	0	0.125	1.72
Samolus valerandi	0 0 0 0 0 0 0 0	0	0 0 0 0 1 0 0 0	0.125	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0.125	0.4
Schoenus nigricans	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	1 0 0 0 0 0 0 0	0.125	0 0 0 1 0 0 0 0	0.125	0.25	0.88
Carex nigra	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 1 0	0.125	0.125	0
Calamagrostis epigejos	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Phragmites australis	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Parnassia palustris	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0
Pedicularis palustris	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0 0 0 0 0 0 0 0	0	0	0

Appendix 9. Results from a nonparametric Tukey multiple comparison. Each species is tested for the three treatments.

Ranks	TREATMENTS	N	day 26 statistics Mean Rank	Tukey multiple comparison NM	BM	KV	day 46 statistics Mean Rank	Tukey multiple comparison NM	BM	KV	day 60 statistics Mean Rank	Tukey multiple comparison NM	BM	HKV	day 75 statistics Mean Rank	Tukey multiple comparison NM	BM	KV	day 79 statistics Mean Rank	Tukey multiple comparison NM	BM	KV
Agrostis stolonifera	No mat (NM)	8	10.625		ns	ns	10.375		ns	ns	9.875		ns	P<0.05	9.8125		ns	P<0.05	9.8125		ns	P<0.05
	De Buiten Muy (BM)	8	8.9375	ns		P<0.05	8.8125	ns		P<0.05	8.5	ns		P<0.01	8.375	ns		P<0.01	8.375	ns		P<0.01
	Het Kapenvlak (KV)	8	17.9375	ns	P<0.05		18.3125	ns	P<0.05		19.125	P<0.05	P<0.01		19.3125	P<0.05	P<0.01		19.3125	P<0.05	P<0.01	
	Total	24																				
Samolus valerandi	No mat	8	14.3125		ns	ns	15.5		ns	ns	15.6875		ns	ns	15.375		ns	ns	15.375		ns	ns
	De Buiten Muy	8	12.625	ns		ns	12.8125	ns		ns	12.625	ns		ns	13.125	ns		ns	13.125	ns		ns
	Het Kapenvlak	8	10.5625	ns	ns		9.1875	ns	ns		9.1875	ns	ns		9	ns	ns		9	ns	ns	
	Total	24																				
Schoenus nigricans	No mat	8	10.75		ns	ns	18.4375		P<0.05	P<0.05	19.125		P<0.05	P<0.01	18.6875		ns	P<0.01	18.6875		ns	P<0.01
	De Buiten Muy	8	12.3125	ns		ns	9.625	P<0.05		ns	10.5	P<0.05		ns	11.125	ns		ns	11.1875	ns		ns
	Het Kapenvlak	8	14.4375	ns	ns		9.4375	P<0.05	ns		7.875	P<0.01	ns		7.6875	P<0.01	ns		7.625	P<0.01	ns	
	Total	24																				
Carex nigra	No mat	8	15		ns	ns	15.8125		ns	P<0.05	15		ns	P<0.05	14.5		ns	ns	14.5		ns	ns
	De Buiten Muy	8	12	ns		ns	14.6875	ns		ns	16	ns		P<0.05	16	ns		P<0.05	16	ns		P<0.05
	Het Kapenvlak	8	10.5	ns	ns		7	P<0.05	ns		6.5	P<0.05	P<0.05		7	ns	P<0.05		7	ns	P<0.05	
	Total	24																				
Calamagrostis epigejos	No mat	8	17.875		P<0.01	ns	19.5		P<0.01	P<0.05	19.8125		P<0.01	P<0.05	20.0625		P<0.01	P<0.05	20.0625		P<0.01	P<0.05
	De Buiten Muy	8	7.375	P<0.01		ns	7.5	P<0.01		ns	7.4375	P<0.01		ns	7.3125	P<0.01		ns	7.25	P<0.01		ns
	Het Kapenvlak	8	12.25	ns	ns		10.5	P<0.05	ns		10.25	P<0.05	ns		10.125	P<0.05	ns		10.1875	P<0.05	ns	
	Total	24																				
Phragmites australis	No mat	8	16.0625		ns	ns	17		ns	P<0.05	16.5		ns	P<0.05	16.375		ns	P<0.05	16.375		ns	P<0.05
	De Buiten Muy	8	11.9375	ns		ns	12	ns		ns	12.9375	ns		ns	13.125	ns		ns	13.125	ns		ns
	Het Kapenvlak	8	9.5	ns	ns		8.5	P<0.05	ns		8.0625	P<0.05	ns		8	P<0.05	ns		8	P<0.05	ns	
	Total	24																				
Parnassia palustris	No mat	8	12.5		ns	ns	12.5		ns	ns	12.5		ns	ns	12.5		ns	ns	12.5		ns	ns
	De Buiten Muy	8	12.5	ns		ns	12.5	ns		ns	12.5	ns		ns	12.5	ns		ns	12.5	ns		ns
	Het Kapenvlak	8	12.5	ns	ns		12.5	ns	ns		12.5	ns	ns		12.5	ns	ns		12.5	ns	ns	
	Total	24																				
Pedicularis palustris	No mat	8	12.5		ns	ns	12.5		ns	ns	12.5		ns	ns	12.5		ns	ns	12.5		ns	ns
	De Buiten Muy	8	12.5	ns		ns	12.5	ns		ns	12.5	ns		ns	12.5	ns		ns	12.5	ns		ns
	Het Kapenvlak	8	12.5	ns	ns		12.5	ns	ns		12.5	ns	ns		12.5	ns	ns		12.5	ns	ns	
	Total	24																				

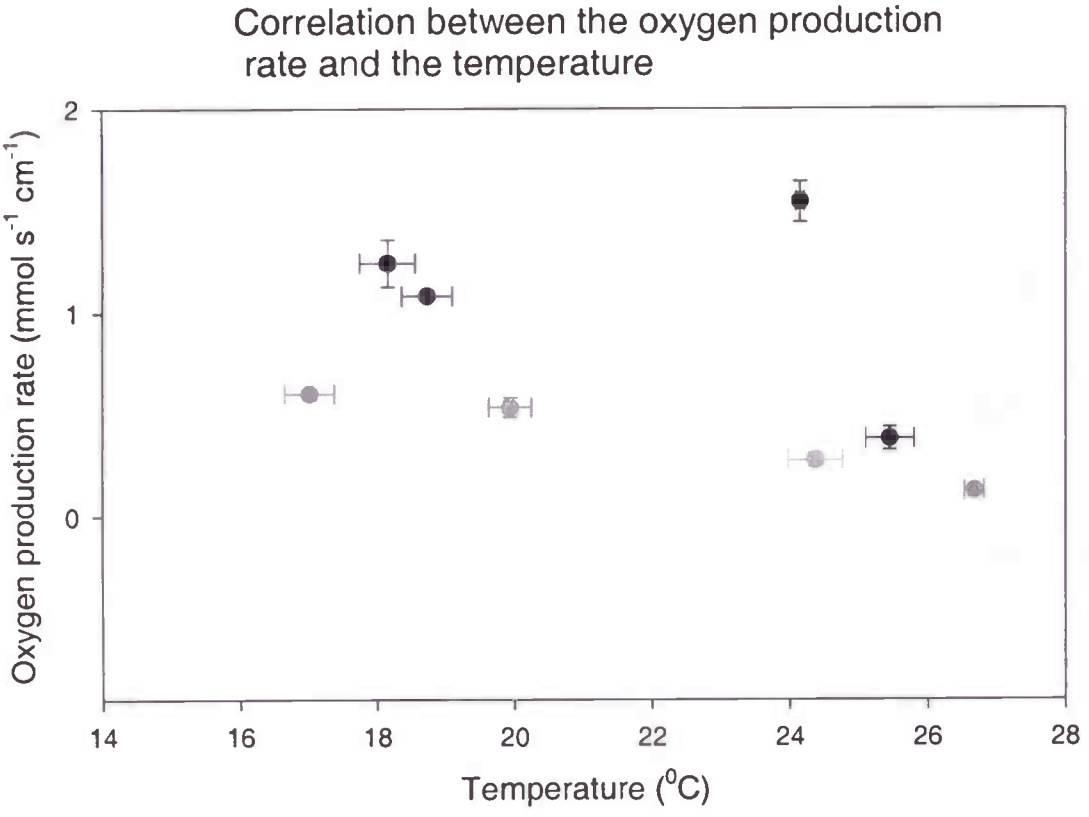
Appendix 10. The nonparametric correlations between the oxygen production rate and the temperature and between the oxygen production rate and the availability of light.

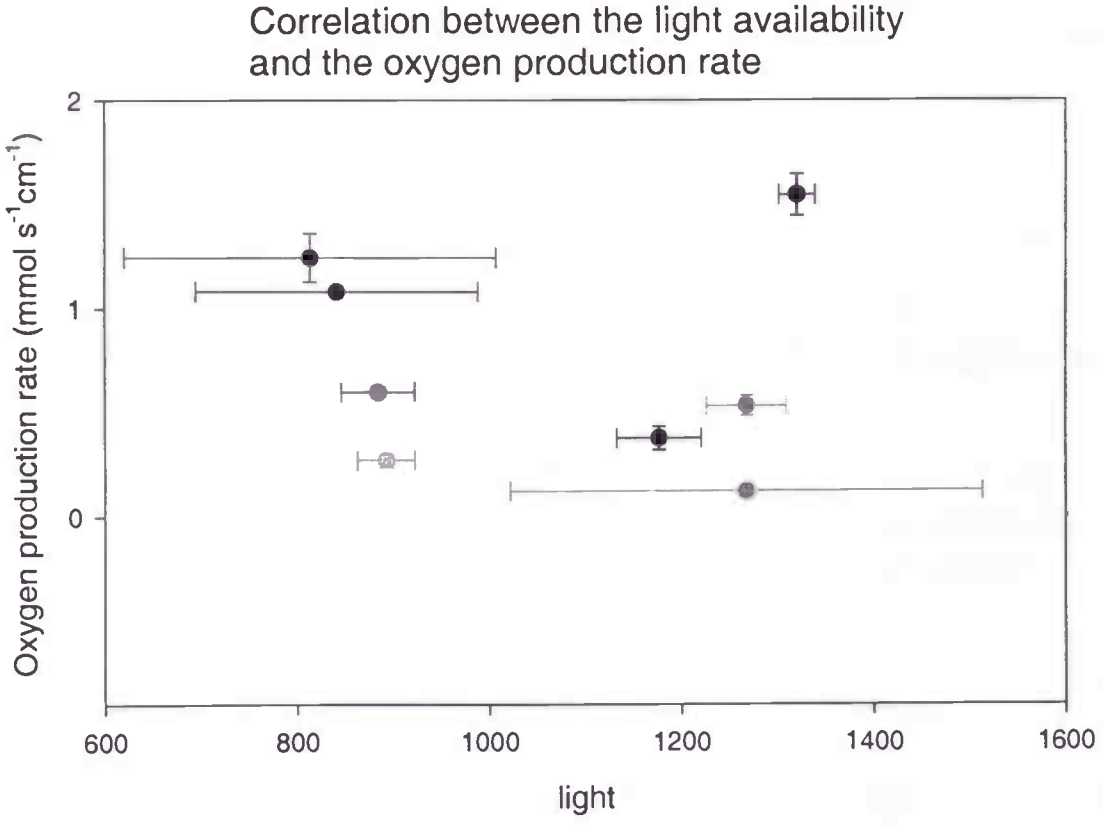
NONPARAMETRIC CORRELATIONS

			RATE	TEMP	LIGHT
Spearman's rho	RATE	Correlation Coefficient		-0.585	-0.088
		Sig. (2-tailed)		0	0.612
		N		36	36
	TEMP	Correlation Coefficient	-0.585		0.445
		Sig. (2-tailed)	0		0.007
		N	36		36
	LIGHT	Correlation Coefficient	-0.088	0.445	
		Sig. (2-tailed)	0.612	0.007	
		N	36	36	

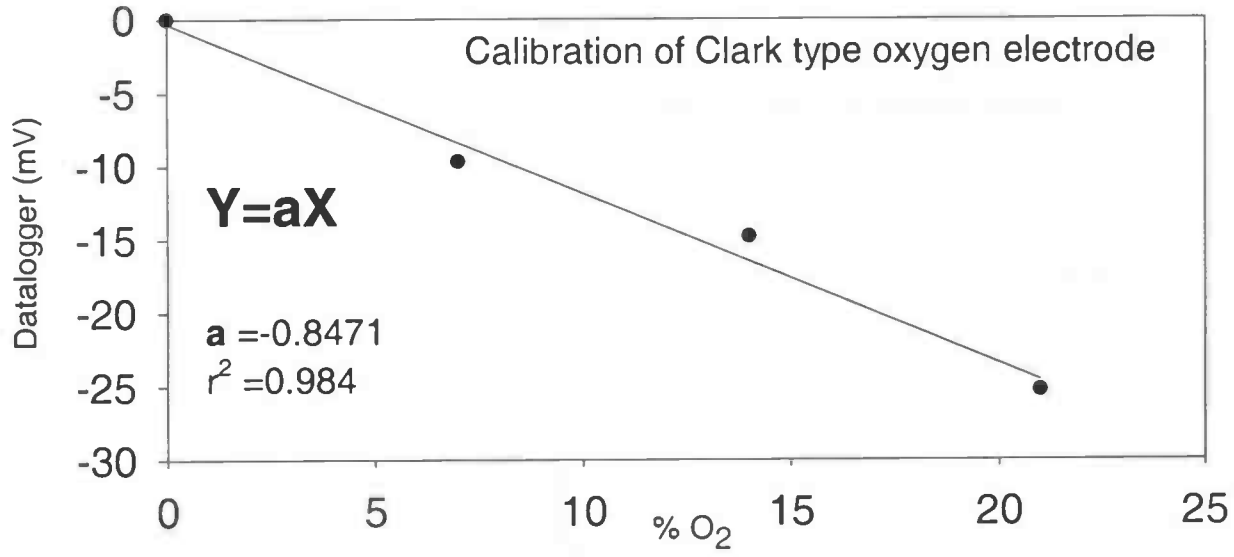
**.Correlation in significant at the .01 level (2-tailed)

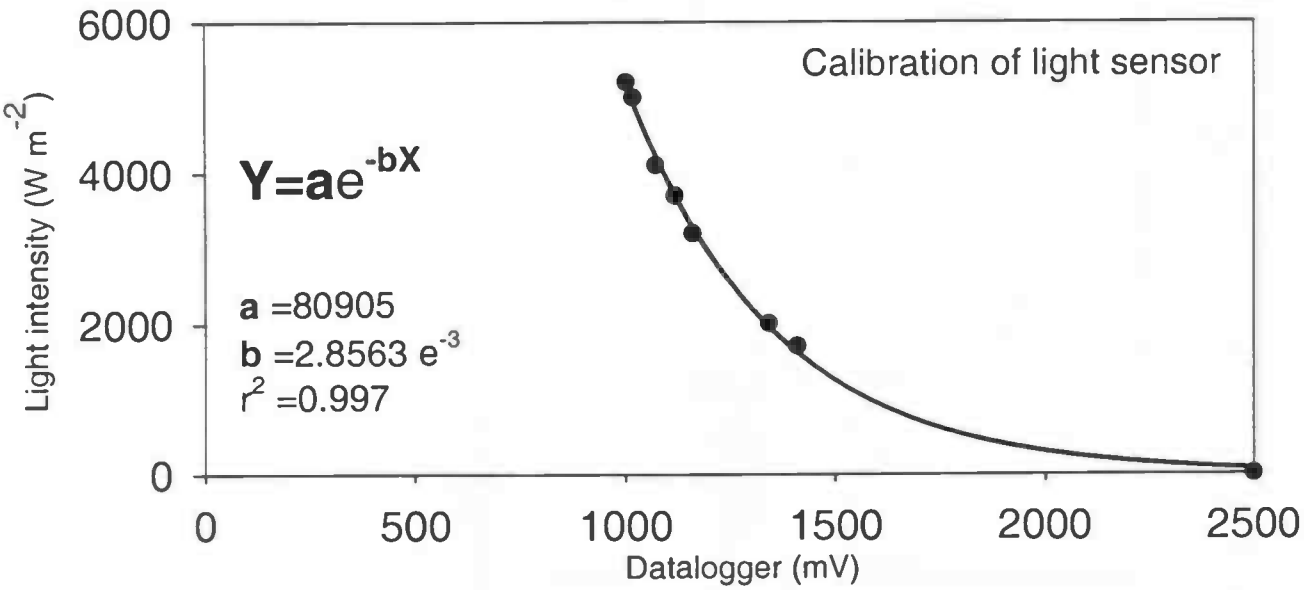
Appendix 11. The graphic present the correlation between the temperature and the oxygen production rate (A) and between the light availability and the oxygen production rate(B).

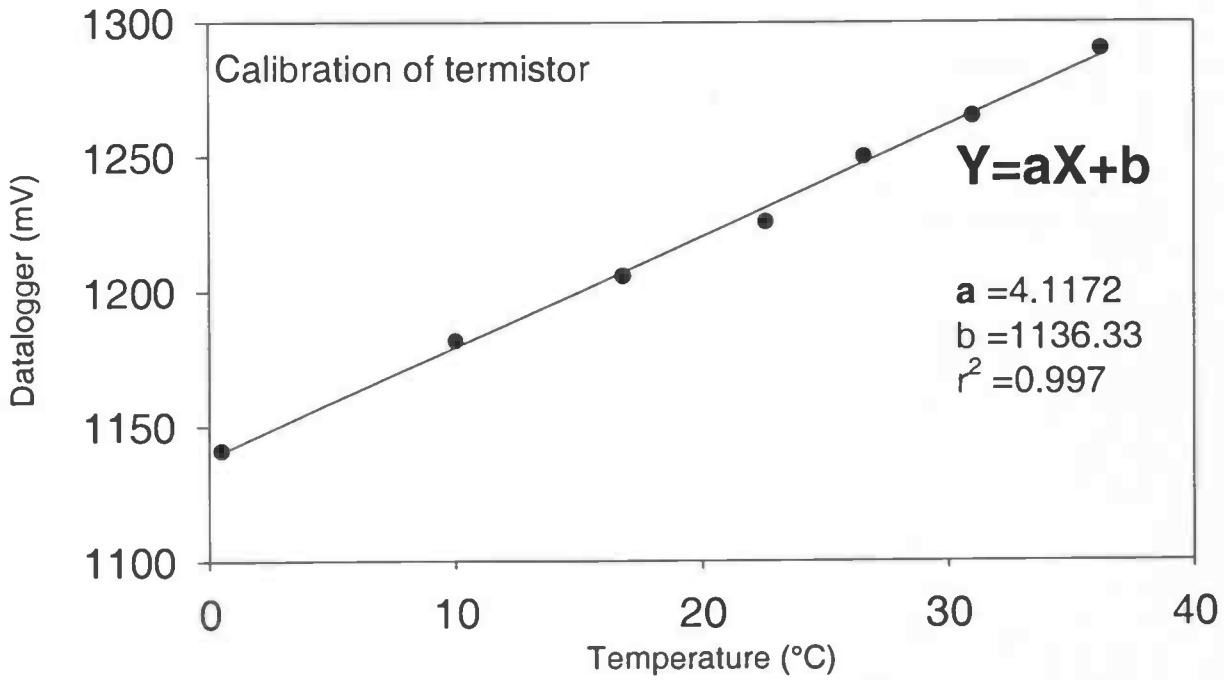




Appendix 12. Calibration of the used sensors.







Appendix 13. **The chamber created by headspace for the field measurements. The oxygen electrode is present in the headspace.**