

Soil algae for combating soil degradation - greenhouse experiment with different soil amendments

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Abstract

Our research contributes to the knowledge of using soil algae to combat soil degradation. We tested green algae (*Klebsormidium flaccidum*) and a field community in a three-month greenhouse experiment and studied their performance on two substrates: sand from the Ordos Desert and artificial desert soil (washed sand). Also a rapid fluorescence microscopy-Image J method was developed to assess the abundance of algae. We studied the effects of four soil amendments (biochar, clay, organic matter, geohumus®) on the development of green algae, field algae and biological soil crusts. *K. flaccidum* developed better than field communities, and field algae preferred washed sand. All amendments had a positive effect on the abundance of *K. flaccidum* and field algae. Biological soil crusts were generally lower in control and organic matter treatments, but relatively higher in biochar and clay treatments. No relationship between algal abundance and soil crust stability was found in this short-term experiment.

1. Introduction

Land is “the terrestrial bio-productive system that comprises soil, vegetation, other biota, and the ecological and hydrological processes that operate within the system” (UNCCD 1994). It is the living environment of human beings, providing ecosystem services such as food, building material, fiber, fuel (MEA 2005). Today, the unreasonable use of land resources leads to the serious soil degradation and desertification of large areas (Reynolds et al. 2007). According to the UNCCD, “desertification means land degradation in arid, semi-arid and dry sub-humid areas resulting from various factors, including climatic variations and human activities”. It is a silent crisis, destabilizing communities world-wide. In the past decades, due to the rapid development of human society, human factors have become increasingly powerful drivers for desertification. These drivers include population growth, mismanagement of land, (e.g. inappropriate use of machinery and chemicals as well as monocultures in intensive agriculture), deforestation, infrastructure, climate change (Reynolds et al. 2007; Zhang and Huisingh 2018; Jiménez-Ballesta et al. 2018).

Desertified land is usually accompanied by physical and biological soil crusts (BSC), particularly the latter being crucial for combating soil degradation (Bowker et al. 2006; Veste et al. 2001). BSC cover 70% of arid and semiarid area (Delgado-Baquerizo et al. 2015), but also are widespread in sub-humid zones (Valentin et al. 2004). Additionally, to soil biota, physical and chemical factors contribute to the formation of BSC (Veste et al. 2001). The highly adapted community of soil biota is composed of green algae, cyanobacteria, bacteria, fungi, lichen, moss, and soil fauna (Lewis and Lewis 2005; Bowker et al. 2008). Green algae and cyanobacteria are pioneers in harsh environments and are found almost everywhere in the soils of terrestrial ecosystems (Tiwari et al. 2019; Hu et al. 2002a).

BSC formed by algae and cyanobacteria play a leading role in erosion control, prevention and mitigation of soil degradation (Hu et al. 2002b; Lababpour 2016). They can improve the soil structure, the stability of the soil surface as well as the content of water and nutrients (Hu and Liu 2003; Swenson et al. 2018; Hu et al. 2002a). During the course of succession, green algae and cyanobacteria are the first colonizers

which contribute to the improvement of soil chemical and physical factors (Eldridge and Greene 1994). Thus, they facilitate the colonization of other organisms which require a more benign environment (Connell and Slatyer 1977; Cole 1990; Weber et al. 2016). Initiating ecological succession is the start of any combat against desertification. Therefore, supporting the establishment and development of BSC is crucial (Bowker et al. 2006; Veste et al. 2001; Park et al. 2017). Artificial spreading of green algae and cyanobacteria was successful in greening part of Ordos Desert (Inner Mongolia). The field algae were isolated, purified and cultured by the Inner Mongolia Academy of Forestry Science Hohhot and then applied to the desert with a water sprayer. The application supported BSC formation with increased organic matter, available N and P providing an improved starting point for the succession of higher plants (Wang et al. 2013). How to help soil algae grow rapidly in desert and form BSC to initiate the ecosystems is the key to current research.

Therefore, we studied BSC formation in a greenhouse experiment using green algae and cyanobacteria with different soil amendments added to desert soil, and developed a rapid method to compare algal growth with the help of fluorescence microscopy. The green algae used were *Klebsormidium flaccidum* (Charophyta), a widespread filamentous dryland species, and the field algae used in this experiment were wild communities on experimental sands at the University of Bremen (Germany), consisting mainly of a mixture of cyanobacteria and green algae including spherical and filamentous *K. flaccidum*. In order to study the growth of soil algae on desert soil, two types of soil matrices were selected, from Ordos Desert and artificial desert soil. In addition, four soil additives were used in this experiment to study their effects on algal growth and soil crust, based on the following research questions (RQ):

1. Is the development of green algae and field community algae different?
2. Is the growth of algae affected by the soil matrix?
3. Which soil amendment has the best effect on the growth of green algae and field community and what is the best quantity of it?
4. Do green algae and field community influence soil pH and BSC stability, which are critical for combating soil degradation?

2. Materials And Methods

2.1 Soil matrices

The Desert sand (DS) was sampled from the DesertDrip testing field at the research station of the Inner Mongolia Academy of Forestry Science, Hohhot (Inner Mongolia, China; Kesel, personal communication) (Fig. 1a). The artificial desert soil (AS) was collected from Bremen Weser riverbank (0-20 cm) and washed to remove all silt and clay particles to simulate the texture of a wind-blown desert soil. After air-drying, the texture of the sands was assessed in four classes by dry-sieving.

2.2 Soil Amendments and Experimental Design

The soil amendments for our experiments included minerals, inert and bioavailable organic matter, and polymeric water super absorbent. The choice of the materials was guided by their easy disposability for practical applications. The quantities of the soil amendments applied to the two sands are shown in Table 2.

1. Biochar (BC) is known as an amendment to Terra Preta, a fertile soil in the Amazon region that was man-made in pre-Columbian times (Glaser et al. 2001). Although carbon itself has low bioavailability, its large active surface allows various biological, chemical and physical interactions, including water retention (Ogura et al. 2016; Ladygina and Rineau 2013; Simms et al. 2020). Our biochar was provided by the Delinat Institute of Ecology and Climate Agriculture (Arbaz, Switzerland). It was produced from lop, grape pomace, wood, miscanthus and other suitable biomass at a pyrolysis temperature of 650°C resulting in particularly effective microporosity. Characterization data were provided by AgroLab Switzerland Ltd. (Agricultural and Environmental Laboratory, Root, Switzerland) (Table 3). A low dose of 10% (BC_L) and a high dose of 30% (BC_H) were used for the experiments, respectively.
2. Clay (Cla) is a material that is effective in cation exchange and water absorption. An easily available commercial Cla was used which also contained some barley straw (3% v/v; pH 7.9). A low dose of 10% (Cla_L) and a high dose of 30% (Cla_H) were used for the experiments, respectively.
3. Organic matter (OM) is a mixture of peat and clay (3:1 v/v; pH 5.8), which contains much more peat than the clay amendment and it was added in experiments at low doses of 10% (OM_L) and high doses of 30% (OM_H).
4. Geohumus® (Geo) is Stockosorb, a cross-linked acrylamide/potassium acrylate copolymer with embedded soil minerals. It is a super absorbent that quickly absorbs excess water and releases it when needed. Specific product information is available on the following website (<https://www.geohumus.com/en/> access 22-01-14). The amount added in the experiment was 1%.
5. Combination (Combi) refers to a mixture of all the four amendments Bio, Cla, OM, and Geo in a ratio of 10:10:10:1 by volume, which was added at 31% in the experiment.
6. Control (Ctr) refers to DS and AS respectively without soil amendments.

The control and different soil treatments were filled into 120 ml flower pots. DS was inoculated with only field algae, while AS was inoculated with both field algae and green algae, resulting in twice as much material as DS. With this design, the different performance of *K. flaccidum* and field algae was tested after application on AS (RQ 1). The effect of the substrate on field algae was tested in DS and AS (RQ 2). In order to obtain information about the function of the soil amendments, all tests were carried out with four soil amendments and Combi, respectively (RQ 3 and 4). The experimental design is detailed in Fig. 2.

2.3 Water holding capacity and pH

For the determination of the maximum water holding capacity (WHC) we followed ISO 11268-2 (ISO, 2012). Soil pH was measured after suspending 10g soil sample (DM, 0.5 cm depth) in distilled water,

following the ISO standard protocol 10390 (ISO 2005), at the beginning and the end of the experiment, which is related to RQ 4. In order to maximize the simulation of the desert soil environment, we tested only the initial soil WHC and pH values without any standardization.

2.4 The algae

2.4.1 Field algae

The field algae used in the experiments were collected from the ReviTec® experimental field at the University of Bremen ReviTal10, Germany (Koehler, 2006) in March (Fig. 1b). The community is dominated by filamentous cyanobacteria, with small amounts of filamentous *Klebsormidium flaccidum* (*K. flaccidum*) and single cell green algae (*Chlorella vulgaris*). Newly collected field algae can be seen under fluorescence microscopy with red areas indicating live field algae (Fig. 1d under daylight, Fig. 1e under fluorescence). We separated the field algae from the sand with a flotation device (Fig. 1c). Briefly, (1) 50g (fresh mass) of soil were given to the flotation device, and then suspended with tap water to separate mechanically algae and soil; (2) Water flow should be neither too strong so that sand is not flushed to the sieve nor too low so that the algae are not separated from the sand. The whole procedure lasts approx. 15 minutes; (3) A 50 µm sieve was used to collect the algae from the effluent. The above steps were repeated until the fresh mass of algae reached 50 g, then they were transferred to the top of the 54 flowerpots using the same procedure as green algae.

2.4.2 Green algae (*K. flaccidum*)

To study the growth of cyanobacteria in field algae in this experiment, we compared them with green algae cultivated in the laboratory. The green algae *K. flaccidum* (*K. flaccidum*) for our experiments were obtained from Goettingen University EPSAG experimental institution (Germany). The algae were cultured in agar with BBM + micronutrient medium + metal solution. After 2 weeks growing on agar, the algae were transferred to liquid BM + SE medium (Bold 1949). Incubation temperature was 25 °C, light intensity was 5000 lux with 16 hr light and 8 hr dark cycling (Fig 1F). After growing in the liquid medium for one month, the green algae were enriched through a filter (50 µm) until 50 g (fresh mass) and then transferred to the top of the flowerpots. To obtain a uniform population of green algae on the soil surface, they were first rinsed with tap water into a 1 L beaker and then transferred to a 200 ml volumetric flask and fixed to 200 mL. Next, they were transferred to a 500 mL spray bottle and the water output of the spray bottle was adjusted to 0.4 ml each time. The green algae were sprayed on the soil surface from the first pot to the end. This procedure was repeated for 5 times so that each pot contained 0.5g fresh mass.

2.5 The greenhouse experiments

The treatments (Table 2) were studied in triplicate independent repetition (n=3). The flowerpots were cultured in the greenhouse for three months with ambient sunlight (from 20th March to 22nd June). The temperature ranged from 20 °C in the night to 28 °C in the day. Soil water content in each treatment was

maintained at 60 % of the WHC for each treatment by weighing the pots twice a week and replenishing the lost water using tap water.

2.6 Assessment of Algal growth and Soil development

2.6.1 Assessment of algal growth by microscopy and Image J

The growth of algae can be assessed by measuring the abundance of the algal cells, weighing the dry weight or testing chlorophyll *a* content (Moheimani et al. 2013). The methods either require pure algae samples, or complex processes. Zhang and Filser (Zhang and Filser 2017) developed a method to quantify algal biomass by using a microsensor to measure photosynthesis and respiration and thereby indirectly measure algal biomass. However, this method is sensitive to the abiotic soil environment and different soil treatments can lead to different results. Therefore, we developed a rapid detection method using epifluorescence microscopy photographs (Olympus BX60 microscope with mercury-vapor lamp was equipped with excitation filter 460–490 nm and U-MWIBA2 emission filter 510–550 nm) combined with Image J software to assess the abundance of algae. Briefly, a cardboard template with three evenly spaced circles was placed over the pots to ensure unbiased selection of sample locations. A laboratory spatula was then used to transfer a small amount of surface soil from each circle location to produce a microscope slide. Next, a drop of water was used to spread the sample with a prepared needle and remove large grains of sand before adding a coverslip. Chlorophyll from living algae was visualized as red autofluorescence in the selected excitation mode. All pictures All photographs were taken at a fixed position on the slide with a magnification of 10×40 for subsequent analysis. The percentage of red parts in relation to the whole field was assessed by the software Image J 1.46r. Firstly, the image was adjusted using color threshold to make sure the red part was selected and make sure to use “Analyze - Set Measurements” to check whether “Area” and “Limit to threshold” are selected, otherwise the measurement is the area of the whole picture. Secondly, the “Analyze Particles” function was used to calculate the area of the red fraction, and finally the “% area” result was derived to estimate the abundance of the algae. Three slides were prepared for each pot and three pictures were formed for image J analysis. The average of the three pictures results was then used as the abundance of algal growth in a single pot.

2.6.2 Soil development

After three months of algal growth in the greenhouse, BSC stability and pH were assessed. A pocket penetrometer (geotest E280) was used to test the mechanical stability of the crust. The penetrometer was inserted into the exactly center of the soil for about 1.5 cm and the value was read (kg/cm^2). After the measurement of BSC stability, soil pH was measured according to the procedure introduced before.

2.7 Statistics and evaluation

Statistical analyses were performed with SPSS 23.0. Data were not transformed. Shapiro-Wilk test ($p > 0.05$) was used to test for normal distribution. Variance homogeneity was analyzed with Levene’s test ($p > 0.05$). General linear model (one-way ANOVA) was used to analyze the main and interaction effect of

treatment and algae as influencing factors; pairwise comparison (Bonferroni) was used to show significant effects between the treatments, soil matrices and the two groups of algae. Spearman correlation was used to analyze the relation between algae abundance, BSC and soil pH. All tests were run with and without outliers, the outliers did not change the results of significance, therefore, outliers were included in all results.

3. Results

3.1 Soil conditions

The texture of AS is coarser than that of DS, which is mainly a medium sand (Table. 1). As expected from the soil texture, WHC of the DS was higher compared to AS with coarser texture (Fig. 3a). The effect of soil texture on WHC was significant. Overall, the WHC was higher for DS than for AS. The different soil amendments differed significantly and were more effective at high doses than at low doses, with OM having the greatest effect on it, significantly increasing WHC for both soil matrices, while clay had surprisingly little effect, with no significant difference compared to Ctr. Combi was the second best amendment for increasing soil WHC, followed closely by BC. Geo, although containing a supersorbent, it was not as effective as the other soil amendments for soil WHC and ranked fourth.

Soil pH was significantly affected by soil amendments, with DS having a significantly lower pH than AS in Ctr. Interestingly, the addition of soil amendments both narrowed the gap between the two, i.e., the pH of DS was generally raised and the pH of AS was generally reduced. A trend towards a higher buffering capacity is observed in the slightly loamy DS, exhibiting a range of pH 0.59 units (from 7.03–7.62) as compared to 0.83 units (from 6.81–7.64) in the silicaceous AS.

3.2 Algal growth, soil pH and crust formation

3.2.1 Algal growth

Figure 4 shows the abundance of field algae and green algae in two soil matrices with different soil amendments. Overall, the growth of green algae was better than that of field algae (14–77% and 12–26%, respectively). In the treatments of BC, Cla and OM, high doses resulted in more pronounced growth of green algae. However, the growth performance of *K. flaccidum* in Geo was worse than the control. The order of the positive effects of these treatments on green algae was BC > Cla > OM > Combi > Ctr > Geo (Fig. 4b). The soil matrix had a significant effect on the growth of algae in the field (Fig. 4a). In BC_L, Cla_H, OM and Ctr, the abundance of field algae was significantly lower in DS than in AS, and in the other groups, there was no difference between each other. The addition of BC_H, Cla_H and Geo increased the abundance of field algae in DS (positive effect: Geo > Cla_H = BC_H), while BC_L, Cla_H, OM_H and Geo had a positive effect in AS compared to Ctr. Combi, on the other hand, had a negative effect on AS.

3.2.2 Influence of algae on soil pH

At the end of the experiment, soil pH is shown in Fig. 5 (a and b) and the change in soil pH over the three months of the experiment was calculated according to the following equation.

$$\Delta\text{pH} = \text{pH}_{\text{March}} - \text{pH}_{\text{June}}$$

The results are shown in Fig. 5 (c and d). A positive ΔpH value indicated acidification of the soil during the three-month period. In general, the pH values of all samples decreased during the months of the experiment. The average final soil pH with green algae ($\text{pH } 6.61 \pm 0.20$) was higher than that of field algae ($\text{pH } 6.29 \pm 0.63$), and in both soil matrices with the addition of field algae, the average pH of DS (6.72 ± 0.09) was higher than the average of AS (6.29 ± 0.06). Among the treatments with the addition of green algae, BC had the highest soil pH, followed by Cla, OM and Combi. However, there was a different story if we calculated the ΔpH . ΔpH was greatest in Ctr, Combi and Geo, while BC, OM and Cla significantly reduced the ΔpH .

In the treatments with field algae, the pH of the soils varied considerably. Firstly, the mean of the final soil pH of DS was significantly higher than that of AS, while the difference was not so consistently significant at the beginning of the experiment. In DS, the treatment groups with the addition of BC, Cla_H, OM and Combi had significantly higher pH than Ctr. While in AS, the treatment groups with the addition of BC_L and Geo exhibited significantly lower pH compared to Ctr. Similarly, we compared the change in pH values ΔpH before and after the experiment and found that in DS soil matrix, Cla_H, Geo and Combi had significantly larger ΔpH values than Ctr, while the opposite result was found in AS that all ΔpH were significantly smaller compared to Ctr except Geo and Combi.

3.2.3 The stability of BSC

The treatments of BC_H, Cla and Combi favored the stability of BSC in both soil matrices, and the BSC in the DS was significantly stronger than that in the AS with the treatments of BC_H and Combi (Fig. 6a). When comparing the effects between green algae and field algae, it was found that BSC was significantly enhanced by the addition of green algae in the treatment of BC_H, while significantly enhanced by the addition of field algae in the treatment of Combi (Fig. 6b).

3.2.4 Correlation of algal growth with soil pH and crust

The Spearman correlation analysis of algal abundance with soil pH and crust stability at the end of the experiment (Fig. 7) showed that irrespective of the treatments green algae abundance was positively correlated with soil pH ($0.749, p < 0.001$) and BSC stability ($0.725, p < 0.001$). The abundances of field algae were negatively correlated with soil pH ($-0.482, p=0.011$) but not with BSC stability ($0.030, p=0.882$).

4. Discussion

In the present study, autofluorescence of chlorophyll was successfully used to assess the abundance of algae and to evaluate growth differences between treatments and soils. We assume that the examination

of three slides per sample compensates for the patchy distribution of the algae in the flowerpots and possible errors associated with sampling, transfer of algae and preparation of microscopic slides.

We used two soil matrices and four amendments for a three-month experiment. At the beginning of the experiment, green algae and field algae were transferred to the pots on the same day. After three months, it was observed that the green algae developed better than the field algae (RQ1). Usually cyanobacteria (that is, the majority of field algae) are more likely to survive than green algae in dry environments that are prone to desertification. Intense solar radiation, high temperatures and drought are tolerated by many species of cyanobacteria (Pluis 1994). Our short-term greenhouse experiments reflected only a small fraction of the real environment that we had moderately wet conditions under which green algae could perform better than cyanobacteria.

The results of soil WHC and pH, algae growth and soil crust experiments are summarized in Table 4. Soil matrices and amendments had important effects on both algal growth and the development of BSC. DS comes directly from the desert, while AS comes from the river bank which is washed before use, so it is coarser and lacks water-removable fractions (silt, clay), resulting in a low buffering capacity (Fig. 3b and Fig. 5a, 5c). In our experiments, we found that field algae showed a strong preference for AS (RQ2). The treatments predominately had positive effects on the development of algae, underlining the potentials of soil amendments in combating desertification.

BC and Cla are generally good promoters of algal growth in the soil. Dosage plays an important role in the growth of field algae that they seem to prefer low concentrations of BC and high concentrations of Cla, while green algae have no significant dose requirements for either additive, with both low and high doses increasing their abundance to varying degrees. In addition, high levels of OM can also increase the abundance of both types of algae in AS. Therefore, we recommend the use of low doses of BC, high doses of Cla and OM to increase algal abundance (RQ3).

There have been only few studies in recent years about BSC in the temperate zone (Dumig et al. 2014; Gypser et al. 2016; Schaub et al. 2019), while most studies have been conducted in arid and semi-arid environments (Li et al. 2002; Budel et al. 2009; Belnap and Gillette 1998; Ghazban et al. 2018). The development of BSCs can generally be divided into four stages: bare soil, algal/cyanobacterial crust, lichen and moss crust (Housman et al. 2006). The BSC studied in our experiment relates to the second stage. The effect of soil texture on BSC is complex (LUND 1945; Pluis 1994; Budel et al. 2009). Hassanzadeh et al. (2019) found that increasing soil moisture and clay content can be favorable for the development of BSC, which is consistent with the results of our study that biochar and clay in the soil can significantly increase the formation of BSC (RQ3). BC had a good performance for the formation of BSC. Although BC is quite inert without any direct fertilizing effects it influences soil physics, and the activation of microflora activity may result in an improvement of the availability of nitrogen and other nutrients, all being supportive for the formation of BSC even in the stage of bare soil (Eldridge and Greene 1994). OM is an important indicator of soil fertility, and high levels of it provide more nutrients for microbial metabolism as well as an increase WHC (Swenson et al. 2018). However, its effect on BSC is more

pronounced in the lichen crust and moss crust stages, where high OM increases the abundance of lichens, fungi and mosses (Schneider et al. 2012). We also found a similar result that OM was associated with higher soil WHC and higher algal abundance. From Table 4 we can see that OM was associated with higher soil WHC and higher algal abundance (only in AS), but not with BSC formation at this very early stage. A similar phenomenon occurred with GEO, which is a water retention agent that can rapidly absorb excess water from the soil and release it when needed; therefore, it can significantly improve soil WHC and field algae growth in this experiment.

Compared to neutral pH of the DS, the initial pH of the AS was slightly alkaline which is preferred by cyanobacteria (Alghanmi and Jawad 2019). This could explain why field algae grow better in the AS. However, the pH of AS decreased more than the DS after three months due to its low buffer capacity. We observed a significant correlation between algae growth and soil pH. Green algae were positively correlated with soil pH, while field algae were negatively correlated with it (RQ4). Soil pH increases due to the photosynthetic assimilation of CO₂ by the algae (Dubinsky and Rotem 1974), which explains why the high abundance of green algae leads to a relative higher pH. The negative correlation between field algae and soil pH may be due to the mortality of field algae, which we observed under the microscope but were unable to quantify. The decomposition of dead field algae may have produced organic acids (Metting 1981) and relevant greenhouse gasses (CO₂, CH₄ and N₂O), which lowered soil pH (Michalak et al. 2016). This observation is consistent with another result that the abundance of green algae was positively correlated with BSC while the field algae were not (RQ4). The complexity of the results of this study clearly shows that effects of soil amendments vary not only by their properties and amounts applied, but also by the soil matrix and the organisms investigated. According to our findings, three months is not long enough for the development of BSC even if we added algae and cyanobacteria as starter. Therefore, the results of this experiment only represent a possibility at the early stage of combating desertification.

5 Conclusions

We compared the growth of green algae and field algae in desert soil and artificial washed sand and the effect of different soil amendments on algal growth and BSC development to provide an experimental basis for combating soil degradation and desertification. Both low doses of biochar and high doses of clay are very helpful for algal growth and BSC development in the early stages of ecological succession. Although high doses of OM are also effective for algal growth, they are not satisfactory for the development of soil BSC. Therefore, here we do not recommend the application of OM in the initial stages of combating desertification. Likewise, the documented performance of Geo does not support its recommendation here. Long-term field studies are mandatory for the further study. Moreover, laboratory and greenhouse studies should include not only the effect of texture and mineral composition of the degraded soils, but other relevant environmental factors in the respective areas, such as soil water content, water holding capacity, factors of wind and water erosion, diurnal and seasonal temperature differences, etc., to simulate the "real world" as much as possible. Studies of biogenic substrate stabilization (Koehler 2006; Koehler and Weidemann 1995) require precise analysis of complex

ecosystems to make scientific recommendations for combating soil degradation, soil erosion and desertification. In addition, we developed a rapid fluorescence microscopy- Image J method for algae growth, which was well applied in this experiment. This method cannot determine the absolute abundance of algae, but can quantitatively compare algae growth between each other under different conditions, and its rapidity and effectiveness can provide a feasible detection solution for future algal growth studies.

Abbreviations

AS: Artificial sand

BC: Biochar

BSC: Biological soil crust

Cla: Clay

Combi: Combination

DS: Desert sand

Geo: Geohumus[@]

K. flaccidum: *Klebsormidium flaccidum*

OM: Organic matter

UNCCD: United Nations Convention to Combat Desertification

WHC: Water holding capacity

Declarations

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Authors' contributions

Both authors contributed to the design of the experiments in this study. XZ performed the practical work and initiated and drafted the manuscript. HK supplemented, revised and commented the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

The datasets and materials (pictures, raw data) used in this study are available from the corresponding author - both upon justified request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare that they have no competing interests.

Tables

Table 1. The texture of the sands assessed by dry sieving

Sieving	DS (%)	AS(%)
Sand (2 mm–630 µm)	0.05	36.45
Medium sand (630–200 µm)	92.85	61.05
Fine sand and coarse silt (200–40 µm)	7.10	2.50
Silt and clay (< 40 µm)	0.00	0.00

Table 2. Summary of the soil amendments applied to the two kinds of sand in the greenhouse experiment.

Sand	Soil amendments	Label	% (v/v)	DS (mL)	AS (mL)	Biochar (mL)	Clay (mL)	OM (mL)	Geo (mL)	Sum (mL)
DS	Biochar	BC_L	10%	450		50				500
		BC_H	30%	350		150				500
	Clay	Cla_L	10%	450			50			500
		Cla_H	30%	350			150			500
	OM	OM_L	10%	450				50		500
		OM_H	30%	350				150		500
	Geo	Geo	1%	495					5	500
	Combination	Combi	31%	345		50	50	50	5	500
	Control	Ctr	0	500						500
AS	Biochar	BC_L	10%		900	100				1000
		BC_H	30%		700	300				1000
	Clay	Cla_L	10%		900		100			1000
		Cla_H	30%		700		300			1000
	OM	OM_L	10%		900			100		1000
		OM_H	30%		700			300		1000
	Geo	Geo	1%		990				10	1000
	Combination	Combi	31%		690	100	100	100	10	1000
	Control	Ctr	0		1000					1000

Table 3. Characteristics of biochar used in the experiment.

Content	Unit	Value
C _{org} [%]	%DM	22.5
C/N	-	18.4
pH	-	7.5
Nitrate-N	g N/t DM	724.6
P	kg P ₂ O ₅ /t DM	7.1
K	kg K ₂ O/t DM	9.1
Ca	kg Ca/t DM	59.2
Mg	kg Mg/t DM	5.4
C _{org} = organic carbon		
DM = Dry Mass		

Table 4. Summary of soil WHC and pH, algal growth, and BSC experimental results. “+” represents increase, “0” represents no significant change and “-” represents decrease compared to Ctr. The gray part represents inoculated with green algae and the white part represents inoculated with field algae (Bonferroni, $p < 0.05$).

			BC_L	BC_H	Cla_L	Cla_H	OM_L	OM_H	Geo	Combi
WHC		DS	+	+	0	0	+	+	+	+
		AS	+	+	0	0	+	+	+	+
Growth of algae	field algae	DS	0	+	0	+	0	0	+	0
		AS	+	0	0	+	0	+	+	-
	green algae	AS	+	+	+	+	0	+	-	+
BSC	field algae	DS	0	+	+	+	0	0	0	+
		AS	0	+	+	+	0	0	0	+
	green algae	AS	0	+	+	+	0	0	0	0
pH_initial		DS	+	0	+	+	0	+	+	+
		AS	-	0	-	-	-	-	0	0
pH_final	field algae	DS	+	+	0	+	+	+	0	+
		AS	-	0	0	0	0	0	-	0
	green algae	AS	+	+	+	+	+	+	0	+
ΔpH	field algae	DS	0	0	0	+	-	0	+	+
		AS	-	-	-	-	-	-	0	0
	green algae	AS	-	-	-	-	-	-	0	0

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Figures

Figure 1

Desert soil and soil algae used in this experiment. a: Location of the DesertDrip testing field in the Ordos Desert, the origin of the Mongolian sand (red circle). b: Field algae collection site, located at the Desert Ecology Experimental Site, University of Bremen, Germany. c: Flotation device for collecting field algae. d: Field algae under 200x microscope, daily light, scale bar of 5 μm . e: Field algae under 200x fluorescence microscope, scale bar of 5 μm . F: Green algae culture device

Figure 2

Illustration of experimental design. a: Different soil amendments were added to the DS and inoculated with field algae. b: Different soil amendments were added to the AS and inoculated with field algae. c: Different soil amendments were added to the DS and inoculated with green algae

Figure 3

Soil WHC (a) and pH (b) at the start of the experiment. Arithmetic means $\pm$ SE (n=3) for each treatment. Significant differences as compared to Ctr are marked by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Differences of WHC between DS and AS are significant for all treatments in a ($p < 0.001$) and marked by the pound sign for treatments in b (# $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$)

Figure 4

The abundances of algae at the end of the experiment. Arithmetic means $\pm$ SE (n=3). (a): Growth of the field algae in DS and AS. (b): Growth of the green algae in AS. Significant differences as compared to Ctr are marked by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Differences of algal growth between DS and AS are marked by the pound sign for a (# $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$). The growth of green algae are all significantly better than that of field algae

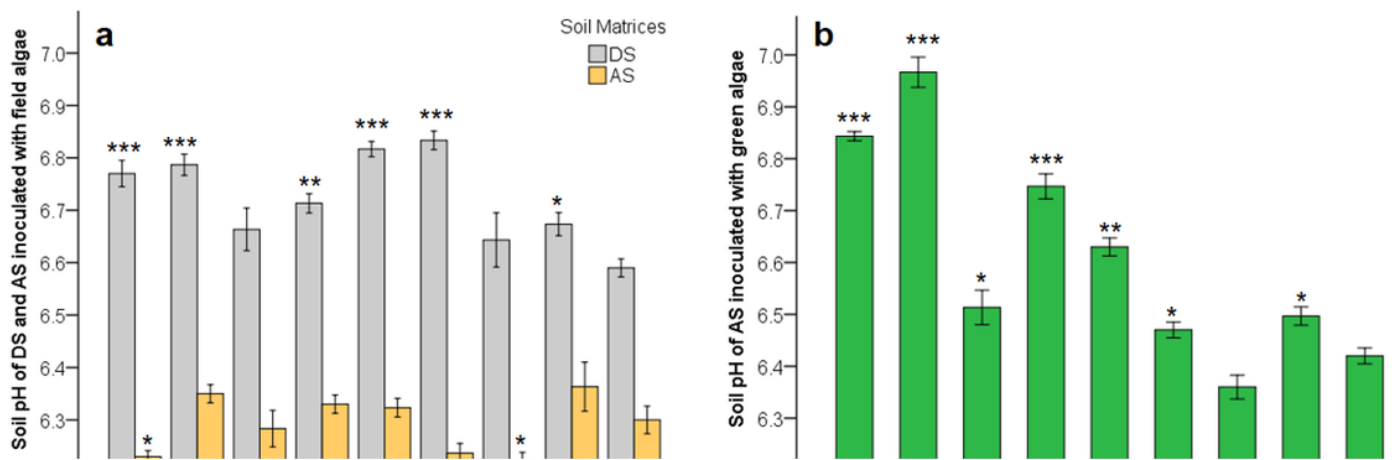


Figure 5

Soil pH (a, b) at the end of the experiment and the Δ pH (c, d) of all treatments. Arithmetic means $\pm$ SE (n=3). Significant differences as compared to Ctr are marked by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Significant differences between soil matrices and algae are observed in all samples for a and b; The pound sign indicates the difference in Δ pH between DS and AS (c) and the difference between green algae and field algae (d, $p < 0.05$)

Figure 6

The stability of BSC after three months of algal growth. Arithmetic means $\pm$ SE (n=3). (a): Effect of soil matrices (DS, AS) on stability of BSC inoculated with field algae. (b): Stability of BSC inoculated with field algae in AS. Significant differences as compared to Ctr are marked by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The pound sign indicates the difference in BSC between DS and AS (c) and the difference between green algae and field algae (d, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$)

Figure 7

Spearman correlation of algae abundance with soil pH and BSC at the end of the experiment. Correlation between (a) Green algae and pH, Correlation Coefficient: 0.749, $p < 0.001$; (b) Field algae and pH, Correlation Coefficient: -0.482 , $p=0.011$; (c) Green algae and crust, Correlation Coefficient: 0.725, $p < 0.001$; (d) Field algae and crust, Correlation Coefficient: 0.030, $p=0.882$

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