The effects of alkalinity and cations on the vitality of *Sphagnum palustre* L.

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SUMMARY

Sphagnum mosses are poikilohydric bryophytes, i.e. dependent on nearly-constant wet conditions. Exposure to mineral-enriched water has long been recognised as a threat to *Sphagnum* mosses and a driver of niche formation. Atrophy of *Sphagnum* is currently attributed to high pH in combination with high calcium concentration. Because the natural occurrence of high pH regularly auto-correlates with alkalinity and calcium concentration it remains unclear which of these factors is detrimental to *Sphagnum*. In a ten-week controlled laboratory experiment we measured the effects of high pH and bicarbonate concentration in combination with various cations (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Fe^{3+}) on K^{+} leakage and survival in *Sphagnum palustre* L. Increased pH (7.2) combined with low ($\leq$ 200 µmol L^{-1}) bicarbonate concentration had no effect. In contrast, high bicarbonate levels (supplied or formed in solution) combined with pH values of 8.0 and higher produced signs of physiological stress (chlorosis and electrolyte leakage) within two weeks and were toxic in all treatments. Cations failed to modulate the adverse effects of high alkalinity; nor could additional potassium alleviate detrimental effects. This study shows that *S. palustre* is adversely affected by increased bicarbonate concentration and alkalinity, which both show a tight correlation with pH and often with calcium levels in water and bedrock. The management of groundwater and surface water sources for restoration of *Sphagnum*-dominated habitats and irrigation of Sphagnum farms should focus on lowering alkalinity levels (including pH), whereas cation concentrations may remain elevated.

KEY WORDS: bicarbonate toxicity, calcium toxicity, paludiculture, peatland, water management

INTRODUCTION

Sphagnum mosses, being poikilohydric, are dependent on the nearly-constant wet conditions which also stimulate peat formation (Clymo 1984, Rudolph & Samland 1985, Rydin & Clymo 1989). The accumulation of carbon in peat occurs when the production of organic matter exceeds decomposition, usually under wet anoxic conditions (Clymo 1984, Hájek & Vicherová 2013). In addition to the quantity of water, the chemical composition of the water has a major influence on *Sphagnum* growth (Sjörs 1950, Kooijman & Bakker 1994, Bragazza *et al.* 2003, Smolders *et al.* 2003). The input of solutes from minerotrophic water may indirectly hamper *Sphagnum* growth by stimulating the growth of vascular plants, triggering vegetation shifts comparable to those observed under high atmospheric nitrogen deposition (Limpens & Berendse 2003, Fritz *et al.* 2014).

Previous work also suggests that *Sphagnum* growth is directly impeded by minerotrophic water (Sjörs 1950, Gorham 1956). Some investigations conclude that the combination of high pH with high calcium (Ca^{2+}) levels is toxic for *Sphagnum* while others mention only one of these two factors as detrimental (Clymo 1973, Smith 1982, Kooijman & Bakker 1995, Vicherová *et al.* 2015). However, the duration of experiments as well as the chemical composition and flow rate of water varies widely between different studies to evaluate the toxic effects of Ca^{2+} (Clymo 1973, Vicherová *et al.* 2015). Interestingly, in very early reports by Paul (1906) and Skene (1915) it is argued that carbonate (CO_3^{2-}) and bicarbonate (HCO_3^{-}), rather than Ca^{2+}, would result in toxic effects in *Sphagnum*.

Although experimental setups vary, in most cases cations are supplied in combination with HCO_3^{-} or hydroxide (OH^{-}). Cations like Ca^{2+} are bound to exchange sites on the cell walls and hydrogen (H^{+}) is released, reacting with HCO_3^{-} to form carbonic acid (H_2CO_3) which results in a lowering of buffering capacity (alkalinity) including pH. Depending on their valencies and concentrations, cations will compete to bind on the cation exchange complex (CEC) of *Sphagnum* (Clymo 1963, Soudzilovskaia *et al.* 2010). The turnover rate of water in contact with the *Sphagnum* can significantly reduce the diffusion

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distances for ions interacting with the cell wall (Clymo 1973, Vicherová *et al.* 2015).

Thus, several abiotic factors influence the CEC, and the exact roles of cations and anions on the vitality of *Sphagnum* remain elusive (Harpenslager *et al.* 2015). Therefore, the following research questions and hypotheses were formulated and tested in a ten-week laboratory study exposing submerged *Sphagnum palustre* to different chemical solutions under light in a climate-controlled growing chamber.

(1) What is the influence of alkalinity (acid buffering) on *Sphagnum* growth? We hypothesised that the addition or formation of HCO_3^- anions, rather than the low concentration of H^+ cations (viz. high pH) in the medium, is toxic to *Sphagnum* mosses.

(2) Do bivalent cations impose higher stress on *Sphagnum* mosses than monovalent cations in an alkaline, HCO_3^- anion rich medium? We hypothesised that HCO_3^- anions or alkalinity will be toxic, independent of the cations (and valency of the cations) accompanying the HCO_3^-.

(3) Do *Sphagnum* mosses respond swiftly (physiological response) or more slowly (growth response) to high alkalinity and/or high cation concentrations and can this be alleviated by adding potassium (K^+)? We hypothesised that rapid changes in electrolyte levels such as K^+ leakage can indicate a primarily physiological response and may be alleviated by addition of K^+ (Demidchik *et al.* 2014).

(4) Can observed toxic effects be reversed by adding iron chloride ($FeCl_3$)? We expected that acidification by iron hydroxide ($FeOH_3$) formation may reduce the negative effects of HCO_3^- anions but may cause iron toxicity in *Sphagnum* (Spratt & Wieder 1987).

METHODS

Sample collection and experimental setup

The *Sphagnum* was collected in January 2017 from two similar stands (located at 52° 26' 43.5" N, 4° 56' 39.0" E and 52° 27' 11.0" N, 4° 55' 47.3" E) on the Ilperveld wetland north of Amsterdam (The Netherlands). *Sphagnum palustre* L. was selected because it occurs in areas with relatively HCO_3^--rich water, although little is known about how extensively or intensively these *S. palustre* habitats are influenced by the surrounding surface water. Other reasons for selecting *S. palustre* were its broad ecological niche, which makes it one of the most common *Sphagnum* species in The Netherlands, and its potential use in Sphagnum farming (Temmink *et al.* 2017, van Diggelen *et al.* 2018, Krebs *et al.* 2018). Furthermore, *S. palustre* is generally found in moist (not flooded) situations having a broad range of acidity but an optimum pH of around 5 (Hájková & Hájek 2004, 2007). For the purposes of this article a high pH is considered to be above pH 7, i.e. exceeding the pH range of *S. palustre* (Wojtuń *et al.* 2013). At such pH levels, inorganic carbon occurs predominantly as HCO_3^-.

After collection, the intact *Sphagnum* mats (thickness >10 cm) were stored outdoors for several weeks, under a shelter to prevent deposition of atmospheric nitrogen and dust. Only the top 3.5 cm (living parts) of the moss shoots were selected for the experiment, to minimise inference from the underlying litter and peat. On average, 9–11 grams of fresh moss material (± 0.3 grams dry material) was taken per sample. The samples were put into polyethylene terephthalate (PET) truncated conical columns (11 cm tall, 7.5 cm diameter) with drainage holes, which were placed in slightly larger polypropylene containers of similar shape but with thinner walls (15 cm tall, 7.5 cm diameter) holding the media (gebr. Kohlmeier GmbH, Vlotho, Germany). The thick rim of the inner container ensured stability of the column, while the drainage holes allowed the experimental solutions to uniformly submerge the moss shoots.

The experiment consisted of ten treatments with five replicates situated in a water bath continuously cooled to 16 °C to minimise evaporation, resulting in a temperature of 18 °C at vegetation level during light conditions. The experiment was conducted in a climate-controlled chamber with a constant temperature of 20 °C and a relative humidity of 55 %. The light regime consisted of 16/8 light/dark periods using LED Toplighting Modules (DR/W/FR_2MB 400V, Philips Greenpower, Poland). The containers received a total PAR (photosynthetically active radiation) of 400 µmol m^{-2} s^{-1} at vegetation level.

All experimental solutions consisted of rainwater solution (Van den Elzen *et al.* 2017; see Table A1 in the Appendix) as a background and the corresponding experimental treatment. Except for the sodium hydroxide (NaOH) treatments, high alkaline treatments received HCO_3^- as sodium bicarbonate ($NaHCO_3$). Sodium chloride (NaCl) was used as secondary control, as a proxy for high cation concentration without pH manipulation (Table 1). Each solution was made by separately dissolving salts in large containers. The solutions were then added to the corresponding conical columns. All solutions were made anew at weekly intervals and

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Table 1. Dominant cations, anions and average pH (means ± SEM) in the last six weeks of the experimental treatments. All treatments consisted of rainwater solution according to van den Elzen *et al*. (2017) (Table A1) plus the experimental treatment.

Treatment	Experimental composition	Cation (μmol L^{-1})	Anion (μmol L^{-1})	pH
Rain	/	/	/	4.8 ± 0.2
NaCl	NaCl	4000	4000	5.1 ± 0.0
NaOH-high	NaOH	2000	2000	11.0 ± 0.0
NaOH-low	NaOH	200	200	9.8 ± 0.1
Na+HCO_3	NaCl $NaHCO_3$	2000 2000	2000 2000	7.5 ± 0.0
Ca+HCO_3	$CaCl_2$ $NaHCO_3$	1000 2000	2000 2000	7.5 ± 0.0
Mg+HCO_3	$MgCl_2$ $NaHCO_3$	1000 2000	2000 2000	7.7 ± 0.0
K+HCO_3	KCl $NaHCO_3$	2000 2000	2000 2000	8.1 ± 0.0
Fe-high+HCO_3	$FeCl_3$ $NaHCO_3$	1400 2000	4200 2000	3.0 ± 0.0
Fe-low+HCO_3	$FeCl_3$ $NaHCO_3$	100 2000	300 2000	7.7 ± 0.0

mechanically stirred until homogenised (n = 5). The treatments were refreshed at weekly intervals. This was done by lifting the column and allowing the *Sphagnum* to drain for at least 30 minutes, during which time the treatment solution in the outer container was sampled and the container was emptied and refilled with 400 ml of fresh treatment solution. This was enough solution to ensure complete submergence of the moss and, thus, full contact of the *Sphagnum* capitula with the treatment solution. A weekly refreshing interval was chosen to avoid substantial HCO_3^- losses, as well as to minimise effects resulting from evaporation. Every week all 50 containers were put into a new fully randomised order.

Prior to the experiment, the *Sphagnum* was acclimatised for a week in 400 ml of rainwater solution. The rainwater solution was also used as a control throughout the experiment (Rain treatment; n = 5). The experiment lasted for 68 days (09 March to 15 May 2017), after which the mosses were harvested. In the first week the experimental solutions were applied at 50 % concentration. This was done because peatlands have a persistent rainwater lens which will initially dilute external inputs. From the second week onwards, the experimental solutions were applied at full strength. Samples were collected from the containers at the end of every week from Week 2 until Week 10 (end of experiment).

Water and moss analyses

At eight of the ten re-suspensions (excluding Weeks 1 and 3) the pH of the solutions in the containers was recorded using a Metrohm 877 Titrino Plus (Metrohm Ltd., Switzerland). Airtight subsamples were taken using a 1 ml syringe for determination of total inorganic carbon (TIC) concentration using an infrared carbon analyser (IRGA; ABB Analytical, Frankfurt, Germany). The pH and HCO_3^- values were averaged over time. Changes in pH and HCO_3^- were determined by subtracting the recorded pH during resuspension from the average pH values in supplied treatment solutions. Additionally, 10 ml subsamples were taken to which 0.1 ml of 65 % nitric acid (HNO_3) was added. These subsamples were stored at 4 °C and later analysed for aluminium (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), total phosphorous (P), total sulphur (S), silicon (Si) and zinc (Zn) using inductively coupled plasma atomic emission spectroscopy (ICP-OES iCAP 6000, Thermo Fischer Scientific, Carlsbad, USA) (Table A2). From Week 2

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until Week 10 the colour of the moss was scored on a scale of zero to four; zero was assigned when the moss was completely green, and four when it was white or brown indicating necrosis/chlorosis (Tsuneda *et al.* 2001).

As a result of stress, in addition to discolouration, cellular membranes may become permeable or even rupture allowing K^+ to leak into solution. Measuring the K content of the mosses allowed us to estimate uptake or adsorption of K^+. The weekly renewal and sampling of the experimental solutions gave initial insights into these processes. Leakage from the mosses was calculated by subtracting the initial inputs from the measured K^+ concentrations in solution. After ten weeks the mosses were harvested dried at 70 °C for 48 hours and homogenised for 3 minutes at 30 hz using a ball mill (Fritsch GmbH, Idar-Oberstein, Germany). 200 mg of dried and homogenised moss material was digested in 4 ml HNO_3 (65 %) and 1 ml hydrogen peroxide (H_2O_2) (35 %) in sealed in Teflon tubes using an Ethos D microwave (Milestone, Sorisole Lombardy, Italy). Digestates were diluted with 100 ml of ultrapure mili-Q water (Milipore corp. Burlington, Massachusetts, USA), after which element concentrations were measured using ICP-OES iCAP.

Statistical analysis

The values displayed in graphs are means with error bars representing 95 % confidence intervals (CI^{95}), while those in Tables are means and standard errors of those means (SEM). Normality was checked using a Shapiro-Wilk test, and for the homogeneity of variance a Levene's test was applied. Data that were not normally distributed were log-transformed. When transformations did not approximate to the assumptions an ANOVA on ranks was utilised (Boos & Brownie 1995). When the assumptions were met, the effects of the different chemical treatments were tested by applying one-way ANOVA. For post-hoc, a Tukey test was used. Treatments were placed as independent factors while discolouration, pH, HCO_3^-, K^+ concentrations in solutions and the K content of moss were placed as dependent variables. Overall averages of the weekly discolouration per container were analysed to justify parametric analysis (Carifio & Perla 2008). Minimum significance was assumed at $p < 0.05$. Initial data exploration and overall analysis were done in R version 3.5.0 (Joy in playing) using the Car package (Fox *et al.* 2018).

RESULTS

Discolouration of *Sphagnum palustre*

At the end of the experiment (after ten weeks) the colour of the *Sphagnum* varied with the treatment ($F(9,40) = 1181$, $p < 0.001$) (Figures 1 and 2). Over the course of the experiment, the moss in Rain and NaCl treatments remained green with only very few white spots ($p = 0.82$). In the NaOH-low treatment it also remained green although with some white spots ($p = 0.01$) (Figure 2). In all HCO_3^- treatments the colour of the moss changed from green to a brownish white ($p < 0.001$) and in the NaOH-high treatment it became completely white within two weeks. In both $FeCl_3$ treatments the *Sphagnum* turned brown ($p < 0.001$), although in the Fe-high+HCO_3 treatment it retained a few faint green spots in the capitulum (Figure 2).

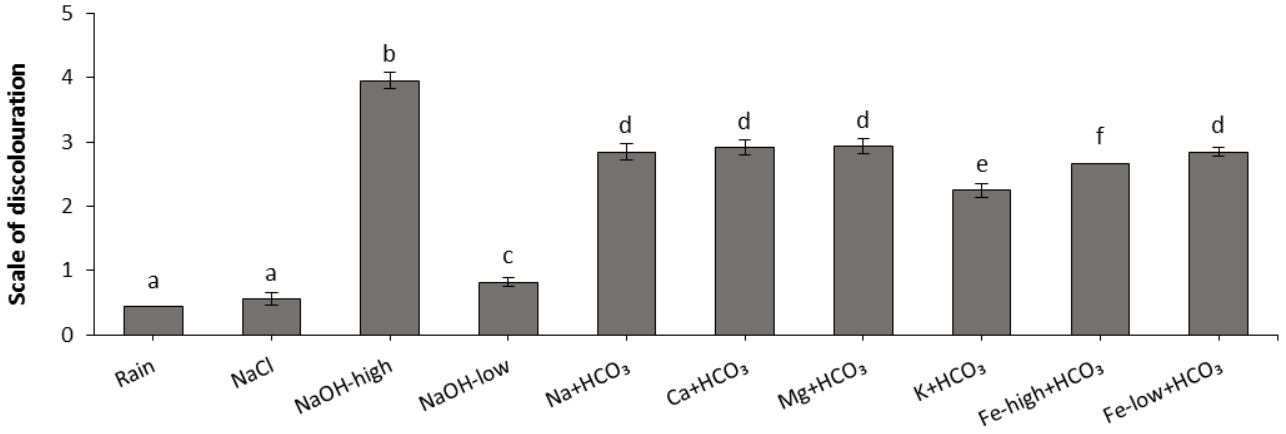

Figure 1. Mean (± CI^{95}, n = 5) discolouration of *Sphagnum* in each treatment over the whole experiment, where 0 indicates no signs of chlorosis or turning brown and 4 indicates full discolouration or loss of natural pigment. Different letters indicate significant differences.

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Changes in pH and HCO_3^- concentration

The average pH over time was significantly different between treatments ($F(9, 390) = 202.1$, $p < 0.001$).

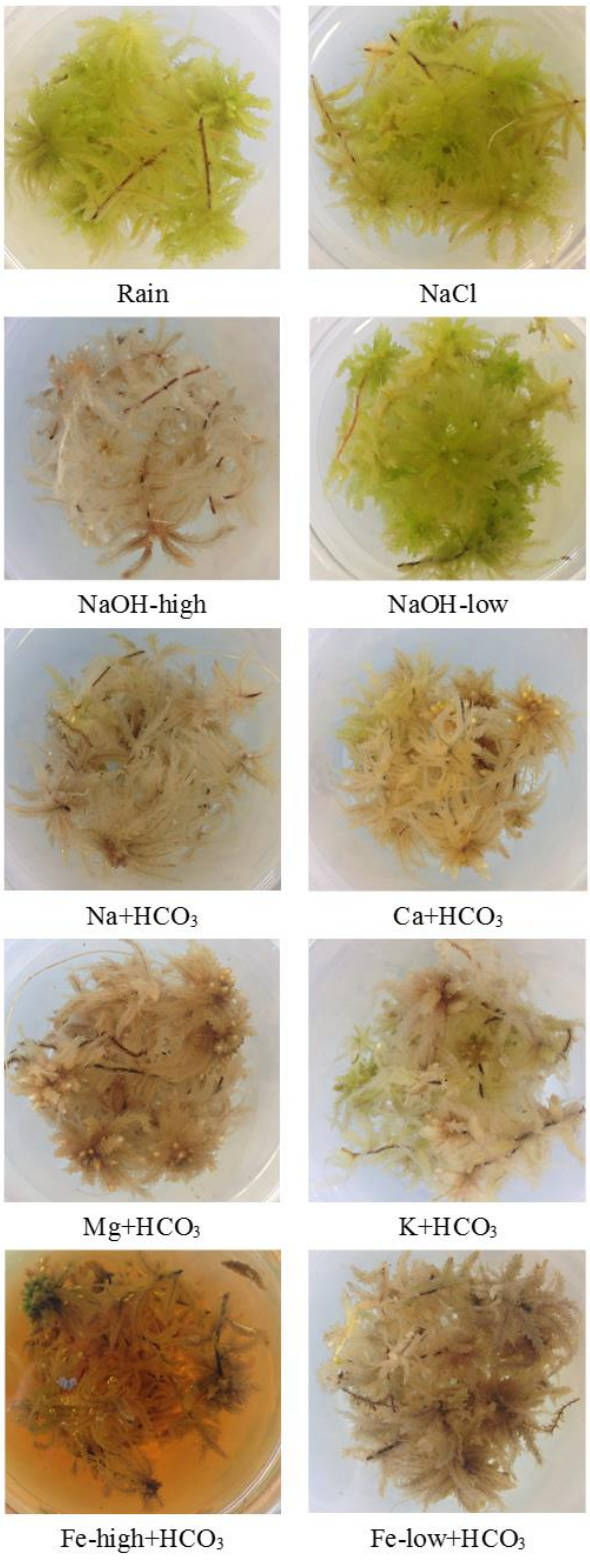

Figure 2. Discolouration effects of the different treatments on *Sphagnum palustre* in the seventh week of the experiment (26 April 2017).

Compared to the Rain treatment, pH was higher in all treatments receiving HCO_3^- except Fe-high+HCO_3 ($p < 0.001$) (Table 2, Figure 3). The NaCl treatment showed a similar pH to the Rain treatment (pH 4.55). The lowest pH (3) was found in the Fe-high+HCO_3 treatment, and this was significantly lower than in all other treatments ($p < 0.001$).

The weekly change in pH also showed differences between treatments ($F(9, 390) = 275.1$, $p < 0.001$). Both NaOH treatments showed, on average, stronger weekly pH decreases than the Rain treatment ($p < 0.001$) (Figure 3). *Sphagnum* subjected to the NaCl and Fe-high+HCO_3 treatments did not show significant differences in pH changes over time compared to the Rain treatment ($p = 0.84$ and $p = 0.52$, respectively). In contrast, all other treatments showed an increase in pH ($p < 0.001$) over time (Tables 2 and A2).

Alongside changes in pH we also found treatment effects on HCO_3^- concentrations ($F(9, 390) = 176.8$, $p < 0.001$). Compared to the Rain, NaCl and Fe-high+HCO_3 treatments, HCO_3^- concentrations were higher in all other treatments ($p < 0.001$). As a consequence of low pH in the Fe-high+HCO_3 treatment HCO_3^- was converted into carbon dioxide (CO_2), whereas in the Rain and NaCl treatments no HCO_3^- was added or formed. We found in-situ formation of HCO_3^- in treatments where only NaOH was supplied. The formation of HCO_3^- also differed

Table 2. Mean (±SEM) water pH and HCO_3^- concentration (mmol L^{-1}) per treatment, for all containers (five replicates) at eight weekly measurements (n = 40). Different letters indicate significant differences ($p < 0.05$).

Treatment	pH	HCO_3^-
Rain	4.5 ± 0^{a}	0 ± 0^{a}
NaCl	4.5 ± 0^{a}	0 ± 0^{a}
NaOH-high	8.7 ± 0.1^{b}	2.02 ± 0.04^{be}
NaOH-low	7.2 ± 0.1^{c}	0.08 ± 0.01^{c}
Na+HCO_3	7.9 ± 0.1^{d}	2.02 ± 0.03^{be}
Ca+HCO_3	8 ± 0^{de}	1.92 ± 0.03^{bd}
Mg+HCO_3	8.1 ± 0^{eg}	2.13 ± 0.04^{e}
K+HCO_3	8.2 ± 0^{eg}	2.11 ± 0.04^{e}
Fe-high+HCO_3	3 ± 0^{f}	0 ± 0^{a}
Fe-low+HCO_3	8.1 ± 0^{g}	1.88 ± 0.03^{d}

between treatments ($F(9, 390) = 44.54$, $p < 0.001$). Over time all treatments in which the dominant added anion was OH^- or HCO_3^- showed an increase in HCO_3^- concentration ($p < 0.01$). In the NaOH-low treatment this significant increase was small (83 µmol L^{-1}). In the NaOH-high treatment, newly formed HCO_3^- reached a similar concentration to that in the experimental HCO_3^- treatments (Tables 2, A2).

Cumulative K^+ changes in solution

Treatments with high HCO_3^- levels (~2 mmol L^{-1}) showed signs of K^+ leakage (Figure 4). Interestingly, mosses in the Rain and NaCl treatments also seemed to lose K^+ to the experimental medium. K^+ leakage was most prevalent during the first 21 days of the experiment. Surprisingly, excess K^+ leakage seemed to be absent in the Ca+HCO_3 treatment. Nonetheless there were strong differences in K^+ leakage between respective groups of experimental treatments ($F(9,40) = 31.9$, $p < 0.001$). Except for the Ca+HCO_3 treatment, all treatments subjected to HCO_3^- and NaOH-high leaked ~100 µmol L^{-1} more K^+ than the Rain treatment ($p < 0.01$). The Fe-high treatment did not differ from the Rain treatment in K^+ leakage ($p = 0.72$). Moreover, the NaOH-low treatment was the only treatment with net adsorption/uptake of K^+.

***Sphagnum* mineral contents**

The amount of K retained by *Sphagnum* varied between treatments ($F(9,40) = 702.8$, $p < 0.001$) (Figure 5). Compared to the Rain treatment, *Sphagnum* receiving high concentrations of OH^- and HCO_3^- retained significantly less K ($p < 0.01$), while the NaCl ($p = 0.33$) and NaOH-low ($p = 0.54$) treatments did not differ in K content. The Ca+HCO_3 and Fe-high+HCO_3 treatments showed a substantial decrease of K (40 % less than the Rain treatment) (Figure 5). Addition of K+HCO_3 resulted in almost double the K content of the Rain treatment ($p < 0.001$).

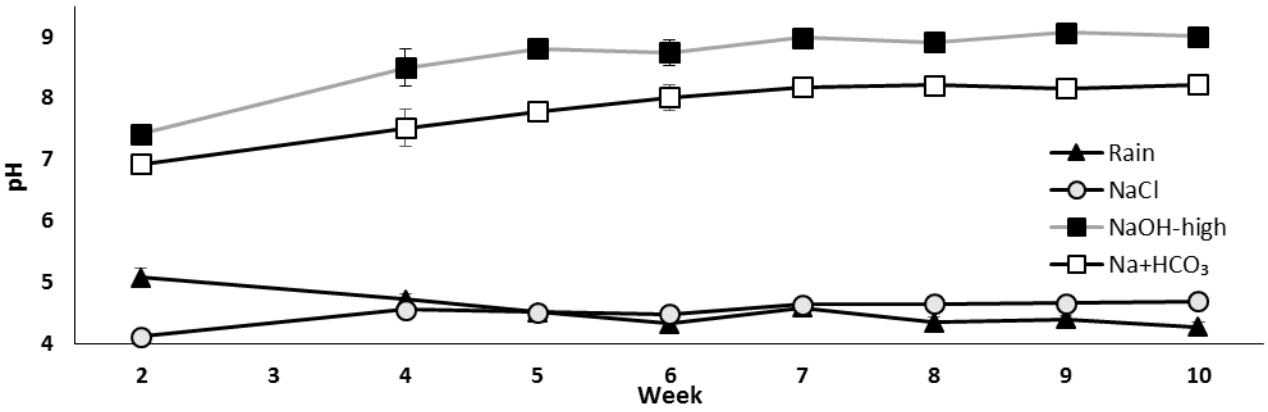

Figure 3. Mean (± CI^{95}, n = 5) pH of the solutions in the containers for four treatments, measured at the end of every week of the experiment.

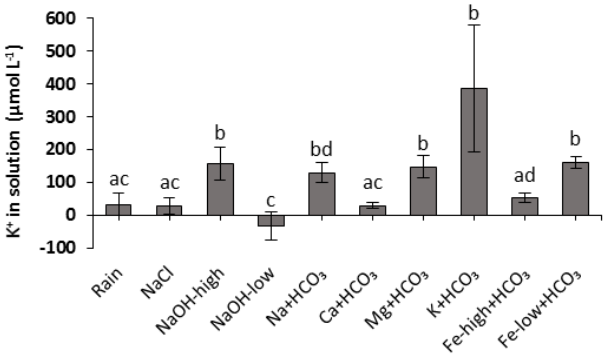

Figure 4. Mean (± CI^{95}, n = 5) cumulative K^+ leakage (µmol L^{-1}) from *Sphagnum* into the treatment solutions with inputs accounted for. Different letters indicate significant differences ($p < 0.05$).

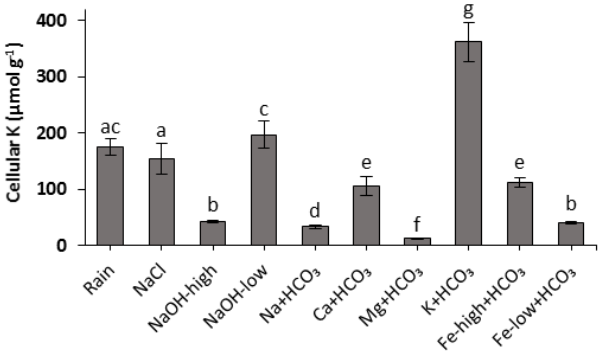

Figure 5. Mean (± CI^{95}, n = 5) dry cellular K contents (µmol g^{-1}) of *Sphagnum* in the different treatments. Different letters indicate significant differences ($p < 0.05$) (Figure 2).

DISCUSSION

Main findings

The exposure of *S. palustre* to high concentrations of added or formed HCO_3^- caused a rapid loss of chlorophyll content (Figure 2) and increased K^+ leakage, indicating impaired membrane integrity. The moss in the NaOH-high treatment was completely white within two weeks, whereas all treatments receiving HCO_3^- started to exhibit adverse physiological effects within three weeks. Interestingly, detrimental effects such as strong chlorosis and K^+ leakage were observed only when high concentrations of HCO_3^- were or became present. All these treatments had mean pH values around 8 so we are not able to distinguish very well between HCO_3^- (alkalinity) and high pH as the main driver for toxicity. In the NaOH-low treatment, however, no adverse effects were detected at pH 7.2. The in-situ formation of HCO_3^- in the NaOH-high treatment was the result of the reaction of (atmospheric) CO_2 with OH^-. There was a consequential lowering of pH from 11 to 8.7 during the incubation. Cations including K^+ failed to modulate the adverse effects of high HCO_3^- concentration and high alkalinity. Furthermore, potentially toxic effects of (specifically) Ca^{2+} cannot be supported by our experimental study, as all combinations of cations and HCO_3^- resulted in *Sphagnum* bleaching.

pH and HCO_3^-

Sphagnum continued to function with addition of 200 µmol L^{-1} NaOH (NaOH-low) despite a high pH (well above 7). In this treatment, in which we found low rates of HCO_3^- formation, *Sphagnum* remained green throughout the experiment and accumulated K^+. Both NaOH treatments provided no initial buffering capacity (Table A1). However, especially in NaOH-high, we measured strong HCO_3^- formation and coinciding atrophy of *Sphagnum*; although there was a methodological constraint in that there was no active flow of the experimental solutions to restrict in-situ formation of HCO_3^-. NaOH-high and/or HCO_3^- treatments had pH values around 8, resulting in relatively low available CO_2 concentrations for photosynthesis which will eventually limit growth in *Sphagnum* mosses (Smolders *et al.* 2001). However, it is unlikely that lack of CO_2 caused the death of the *Sphagnum*. At pH 8 and a HCO_3^- concentration of 2 mmol L^{-1}, CO_2 concentrations were still considerably higher (40–50 µmol L^{-1}) than in the NaOH-low treatment with pH 7.2 and 80 µmol L^{-1} of HCO_3^- (±10 µmol L^{-1} CO_2) (Tables A2, A3). Nevertheless, *Sphagnum* survived the NaOH-low treatment whereas it bleached in the high alkalinity treatments. Of course the weekly subjection to atmospheric CO_2 when refreshing the experimental media might have helped to maintain photosynthesis. *Sphagnum* is also capable of acidifying the HCO_3^- formed in situ and thus producing CO_2 near the leaves which could be utilised for photosynthesis. However, we have no measurements of internal pH in close proximity to the plants.

Sphagnum in treatments receiving HCO_3^- exhibited the same atrophic chlorotic tendency as *Sphagnum* subjected to NaOH-high. These results suggest that adverse effects of high pH require buffering (high alkalinity) provided by the presence of HCO_3^-. The experimental work by Vicherová *et al.* (2015) allows a similar conclusion. Li *et al.* (2018) also speculated about the particular role of HCO_3^- regarding potential adverse effects on *Sphagnum*. Skene (1915) evaluated investigations by previous workers such as Paul (1906) as to why *Sphagnum* degrades so readily when submerged with $CaCO_3$ in solution, and seems inclined to assign the injurious effects of $CaCO_3$ to HCO_3^- or CO_3^{2-}. Clymo (1973) proposed that high pH and high Ca^{2+} concentration are not toxic to *Sphagnum* independently, yet when combined they are. Our results indeed indicate that pH alone is not toxic but the formation or addition of HCO_3^- is. Therefore, it is likely that HCO_3^-, rather than pH itself, predetermines the distribution of bog and poor fen *Sphagnum* species (Vicherová *et al.* 2015, Plesková *et al.* 2016).

In natural systems a high pH is usually associated with a large buffering capacity that prevents acidification, which is typically found in mineral-poor environments like bog and poor fen. The NaOH treatments applied within this experimental study are not representative of natural environments. During the weekly incubations, pH was higher in the NaOH-high treatment (8.7–11) than in the HCO_3^- treatment (7.5–8.2). Interestingly, the mosses in the NaOH-high treatment bleached a week earlier than in the HCO_3^- treatments, although the final HCO_3^- concentrations were comparable. This suggests that the detrimental effect of high buffering capacity is somehow related to an interference with the internal pH regulation of the moss. This will be a subject of further investigation.

Cations and HCO_3^-

No indications of additional stress originating from the combination of different cations with the alkaline HCO_3^- rich solution were observed. Moreover, *Sphagnum* suffered no chlorotic effects of Na^+ in the NaCl treatment (Figure 1). Our results imply that 4000 µmol L^{-1} Na^+ is not toxic for *Sphagnum* within

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the duration of the experiment. This is in accordance with Paul (1906) who placed the lethal concentration of NaCl at 300 mg L^{-1} (7500 µmol L^{-1}), as later confirmed by Wilcox (1984) and Pouliot *et al.* (2013). Adverse effects observed in NaOH-high and Na+HCO_3 treatments are thus likely to be due to the resulting high alkalinity.

All other treatments subjected to different cations in combination with HCO_3^- showed similar extents of atrophy, independently of the cation present. Outcomes were similar for Mg+HCO_3, K+HCO_3 and Ca+HCO_3, as all moss samples died during the experiment. Treatments including K^+, magnesium (Mg^{2+}) and Ca^{2+} without HCO_3^- but having low quantities of OH^- for pH control were lacking in our experiment. However, recently conducted experiments produce results similar to those for NaCl when testing calcium chloride ($CaCl_2$) on seven *Sphagnum* species (Koks *et al.* unpublished data). Despite differences in experimental design, Vicherová *et al.* (2015) also show that $CaCl_2$ additions up to 2.4 mmol L^{-1} do not seem to directly harm *Sphagnum*, whereas HCO_3^- has large negative effects as shown in the present study. For natural environments, however, a persistently high pH may indicate the presence of both HCO_3^- ions and Ca^{2+} (+ Mg^{2+}) ions and therefore be an important indicator for alkalinity stress.

Physiological stress in *Sphagnum*

Sphagnum exposed to high concentrations of HCO_3^- leaked K^+ throughout the experiment, indicating cellular stress (Demidchik *et al.* 2014). This was accompanied by a significantly lower net retention of K (Figure 5). It is likely that the large net retention of K in the K+HCO_3 treatment is due to a large uptake of K^+ in Week 1, which we did not measure and thus excluded from the interpretation. Moreover, leakage was predominantly high in the first 2–4 weeks when most of the K^+ was leaked into the solution. Interestingly we also measured a continuous gradual increase in both pH and HCO_3^- in treatments receiving HCO_3^-, probably due to *Sphagnum* losing acidification capacity following excessive physiological stress (Figure 2). Although these processes coincide with *Sphagnum* bleaching, the exact mechanism remains elusive.

Remarkably, *Sphagnum* in the Ca+HCO_3 treatment leaked less K^+ compared to other treatments receiving HCO_3^-, although it retained 40 % less K than in the Rain treatment. Nevertheless, the moss showed severe chlorosis/bleaching indicating heavy physiological stress (Figure 2). It is well known that Ca^{2+} plays an important role in controlling membrane structure and structural integrity by binding to phospholipids and thus stabilising lipid bilayers (Hepler 2005). This might have resulted in less leakage of K^+ in the $CaHCO_3$ treatment. In any case, our results reveal that K^+ leakage very probably results from decreased vitality and not the other way around. Although monitoring K^+ leakage is not a well-established method within *Sphagnum* research, it has been applied in other experiments using *Sphagnum* (Fritz *et al.* 2012). This method deserves further investigation as it seems to have potential as an indicator for physiological stress in *Sphagnum*.

Reducing HCO_3^- toxicity with Fe^{3+} acidification

Addressing the fourth and last hypothesis, both of the iron (Fe^{3+}) treatments involved adding $FeCl_3$ plus 2000 µmol L^{-1} Na+HCO_3 (Table 1). $FeCl_3$ causes acid conditions (hydrochloric acid (HCl) is produced when $FeCl_3$ dissolves) while HCO_3^- does the opposite. As a result we measured very low pH (3) in the Fe-high+HCO_3 treatment meaning that no HCO_3^- was present, in contrast to the Fe-low+HCO_3 treatment. The Fe-high+HCO_3 treatment retained more K than the Fe-low+HCO_3 treatment. Similarly, compared to the Rain treatment, no significant increase of K^+ leakage was measured in the Fe-high+HCO_3, treatment although this treatment showed significantly lower *Sphagnum* K contents (Figure 4). Spratt & Wieder (1987) discussed Fe^{3+} toxicity occurring in *Sphagnum* due to a solute cation deficiency resulting from the CEC of *Sphagnum* becoming saturated by Fe^{3+} (Clymo 1963). Thus, the high Fe^{3+} concentrations resulting from the Fe-high+HCO_3 treatment probably affected the *Sphagnum* mosses in a different way than the high alkalinity treatments. This also indicates that certain cations, such as Fe^{3+}, can be toxic to *Sphagnum* at low pH.

Implications for management

In natural environments, high pH plus high Ca^{2+} (other cations) and HCO_3^- concentrations (alkalinity) occur in concert. This study helps us to understand which components of well-buffered alkaline water may impede *Sphagnum* growth. Our results indicate that the addition of large quantities of cations such as Na^+ does not alone impose physiological stress on *Sphagnum*; nor does increased pH (7.2) with low HCO_3^- concentration (alkalinity) cause stress. However, substantial quantities of added or formed HCO_3^- provoke substantial stress, preventing growth. With some caution we can conclude that it is not Ca^{2+} in combination with high pH but, rather, high alkalinity (high pH in combination with high HCO_3^- concentration) that has a detrimental effect on

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Sphagnum mosses. Thus, in the context of water management for restoring *Sphagnum* dominated vegetation and Sphagnum farming, it is important to focus on adjusting alkalinity rather than cation concentrations and/or solely pH.

Field methods for lowering the alkalinity of water that should be tested range from biological controls (e.g. root proton release) to technical methods such as the addition of (organic) acids to adjust the inorganic carbon equilibrium. It would also be interesting to determine which designs/vegetation treatments in wetlands can effectively lower the alkalinity of irrigation water before use. The results reported here suggest that cation concentrations may remain elevated as long as solute inputs remain within the physiological range of *Sphagnum* species (Fritz *et al.* 2012, Limpens *et al.* 2012, Chiwa *et al.* 2018) and vascular plants are controlled (Gaudig *et al.* 2018). Fortunately the reduction of HCO_3^- in water is much easier to achieve than the lowering of cations such as Ca^{2+}.

We concede that continuous submersion of *Sphagnum* is an extreme situation experienced in nature by only a few species thriving in acid bog pools which are, therefore, unlikely to be exposed to alkaline-rich waters. However, the findings of the present study may have implications for Sphagnum farming irrigation systems, *Sphagnum* growing reactors and *Sphagnum* restoration projects. Removing HCO_3^- in the low millimolar range may be rather more feasible with the help of HCl instead of $FeCl_3$. We also underline the importance of successful lawn formation in both restoration and farming of *Sphagnum* mosses. A 10 cm thick *Sphagnum* lawn provides acidification potential and will reduce the probability of flooding, thereby lowering exposure to HCO_3^--rich water. Secondly, different *Sphagnum* species occur along a large gradient from mineral-rich fen to ombrotrophic mineral-poor bog (Hájek *et al.* 2006, Rozbrojová & Hájek 2008, Plesková *et al.* 2016, Vicherová *et al.* 2017). Testing the tolerance of these species in light of the results presented here could contribute significantly to improving the effectiveness of restorative/paludicultural management strategies by providing a basis for choosing a set of well-adapted *Sphagnum* species specific to the ecosystem.

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AUTHOR CONTRIBUTIONS

The experiments were conceived, designed and performed by AJPS, CF and GvD. The data were analysed by AHWK, CF, GvD and LPML. LPML also contributed reagents, materials and analysis tools. This article was written by AHWK, CF and GvD with contributions from AJPS and LPML.

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Appendix

Table A1. Chemical composition of the rainwater solution, as used by van den Elzen *et al*. (2017).

	Concentration (µmol L^{-1})	Molecular weight (g mol^{-1})	Concentration (g L^{-1})
Sea salt (wiegand)			5.0E-03
KCl	30.0	74.6	2.2E-03
$CaCl_2 \cdot 2H_2O$	10.2	147.0	1.5E-03
Fe-EDTA	10.1	345.1	3.5E-03
KH_2PO_4	1.0	136.1	1.4E-04
$ZnSO_4 \cdot 7H_2O$	0.7	287.5	2.0E-04
$MnCl_2 \cdot 4H_2O$	0.8	197.9	1.6E-04
$CuSO_4 \cdot 5H_2O$	0.2	249.7	5.0E-05
H_3BO_3	0.8	61.8	4.9E-05
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.008	1236.0	9.9E-06

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Table A2. Chemical composition (in µmol L^{-1} except for HCO_3^- in mmol L^{-1}) of the experimental treatment solutions before addition to the columns each week. Dissolved nitrogen species were not measured. The values are means (± SEM) for Weeks 2–10 (n = 9) except for HCO_3^- (n = 8), CO_2 (n = 8) and Fe-low+HCO_3 (n = 8).

	Rain	NaCl	NaOH-high	NaOH-low	Na+HCO_3	Ca+HCO_3	Mg+HCO_3	K+HCO_3	Fe-high+HCO_3	Fe-low+HCO_3
HCO_3^-	0±0	0.01±0	0.03±0	0.02±0	1.88±0.1	1.87±0.1	1.88±0.1	1.97±0	0±0	1.87±0.1
CO_2	13±4	8±3	0±0	0±0	112±13	194±26	124±30	43±3	1277±113	85±11
Al	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	4±0	0±0
Ca	12±0	12±0	11±0	12±0	12±0	920±114	15±1	12±1	14±1	12±0
Fe	13±0	10±0	9±0	9±0	9±0	9±1	9±0	9±1	1382±22	63±3
K	29±1	29±1	32±1	30±1	29±1	30±1	29±1	2051±60	31±2	29±0
Mg	1±0	1±0	1±0	1±0	1±0	1±0	952±56	3±0	3±0	1±0
Mn	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	5±0	1±0
Na	11±1	3933±75	1968±42	210±2	3968±56	2051±14	2041±32	2074±57	2033±26	1984±13
S	2±0	2±0	2±0	2±0	2±0	2±0	2±0	2±0	6±0	0±0
Si	4±1	4±1	4±1	4±1	4±1	4±1	4±1	4±1	4±1	2±1
Zn	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	4±0

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Table A3. Chemical composition (in µmol L^{-1} except for HCO_3^- in mmol L^{-1}) of the experimental solutions after 7 days of exposure per treatment. Dissolved nitrogen species were not measured. The values are means (± SEM) for Weeks 2–10 (n = 45) except for HCO_3^- (n = 40) and CO_2 (n = 40).

	Rain	NaCl	NaOH-high	NaOH-low	Na+HCO_3	Ca+HCO_3	Mg+HCO_3	K+HCO_3	Fe-high+HCO_3	Fe-low+HCO_3
HCO_3^-	0±0	0±0	2.02±0.04	0.08±0.01	2.02±0.03	1.92±0.03	2.13±0.04	2.11±0.04	0±0	1.88 ±0.03
CO_2	24±3	23±3	31±3	11±1	119±2	47±7	42±2	39±1	23±1	49±2
Al	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	5±0	0±0
Ca	5±0	9±0	9±0	4±0	3±0	898±42	17±1	3±0	16±0	3±0
Fe	8±0	7±0	7±0	8±0	7±0	7±0	7±0	8±0	1400±20	17±1
K	33±2	32±2	49±5	28±1	43±4	33±1	45±3	2110±13	37±1	47±3
Mg	1±0	3±0	1±0	1±0	1±0	2±0	988±23	2±0	4±0	1±0
Mn	0±0	1±0	1±0	1±0	0±0	1±0	1±0	0±0	6±0	0±0
Na	44±28	4210±31	2049±21	165±5	4227±30	2113±12	2155±14	2090±14	2155±25	2140±14
P	0±0	0±0	2±0	0±0	1±0	0±0	0±0	1±0	0±0	1±0
S	1±0	1±0	4±0	2±0	3±0	1±0	2±0	3±0	6±0	3±0
Si	3±0	3±0	4±0	3±0	4±0	4±0	4±0	4±0	3±0	3±0
Zn	0±0	0±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0

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