Sphagnum response to nitrogen deposition and nitrogen saturation – a meta-analysis

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Abstract

Background: *Sphagnum* plants are sensitive to nitrogen deposition changes. However, previous literature is inconclusive on the response of *Sphagnum* species to nitrogen deposition, little is known about the nitrogen saturation of *Sphagnum* growth, and the effects of climatic, spatial and fertilization variables on the response of *Sphagnum* plants are still unclear.

Methods: We conducted a meta-analysis of 66 experiments on nitrogen application to *Sphagnum* plants. The combined effect size of a random-effects model was used to analyze the effect of nitrogen deposition on *Sphagnum* growth. A mixed-effects model was established to determine the significance of each factor for the response of *Sphagnum* plants to nitrogen deposition. The nitrogen saturation value for *Sphagnum* species was obtained by fitting the regression function.

Results: *Sphagnum* growth is promoted by moderate nitrogen deposition, and when nitrogen deposition exceeds the saturation value, *Sphagnum* growth is inhibited. The current nitrogen deposition saturation value for *Sphagnum* species is $19.34 \text{ kg ha}^{-2} \text{ yr}^{-1}$ globally and varies in different latitudinal zones. When *Sphagnum* plants are affected by excessive nitrogen deposition, low-water-table microhabitats and excessive temperature and precipitation can all exacerbate *Sphagnum* growth inhibition.

Conclusions: Excessive nitrogen deposition will inhibit *Sphagnum* growth, and the nitrogen saturation values we obtained for *Sphagnum* species can be used to adjust fertilizer applications to protect *Sphagnum* plants from excessive chemical fertilizer application.

Introduction

Sphagnum is a dominant genus of peatlands and the decomposition of *Sphagnum* litter is extremely slow. The rate of *Sphagnum* productivity far exceeds the decomposition rate, thus resulting in the fixation of large amounts of carbon in peatlands (Rydin, 2006). Peatlands are important global carbon sinks, with more than half of the carbon sequestration worldwide since the Holocene being performed by *Sphagnum* species (Gorham, 2012; Freeman, 2012). *Sphagnum* plants lack a root system and vascular tissue, and atmospheric inputs are their primary source of nutrients, making them sensitive to changes in atmospheric nitrogen deposition (Bobbink, 2003). Since the industrial revolution, the burning of large amounts of fossil fuels by factories, the high emissions of nitrogen oxides as a result of urban construction, and the application of large amounts of nitrogen fertilizers to agricultural fields has led to increased atmospheric nitrogen concentrations and deposition in peatlands via rainfall and dust (Galloway et al, 2004; Erisman et al, 2011; Miriam, 2014). In recent decades, the area coverage of *Sphagnum* has greatly declined, and *Sphagnum* growth has been restricted; a rapid increase in nitrogen deposition is considered one of the possible causes (Gerdol, 2008; Sheppard, 2013; Juutinen, 2016; Shi XM, 2017; Wieder, 2019). It has been shown that increased nitrogen deposition may lead to a shift in the competitive balance in favor of vascular plants and decrease *Sphagnum* growth, thus reducing the rate of carbon sequestration in peatland ecosystems (Limpens, 2006). Breeuwer (2009) and others have also

shown that a high nitrogen application has a negative effect on *Sphagnum* production, particularly at lower latitudes. Some studies have also shown that increasing nitrogen deposition promotes *Sphagnum* growth (Bonnett, 2010; Vitt, 2003), and Berg A (2013) et al. found that increased nitrogen levels explained 76% of the increase in *Sphagnum* biomass. However, an experiment conducted by Gaudig G (2020) et al. showed no effect of continued nitrogen fertilizer application on *Sphagnum* growth under high-nitrogen-deposition conditions ($38 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$). Overall, there have been no uniform conclusions about the reaction of *Sphagnum* to changes in nitrogen deposition among existing studies.

The conclusions about the response of *Sphagnum* to nitrogen deposition have varied among studies, which may have been due to the different factors considered. Previous literature has shown that precipitation, temperature, nitrogen deposition, and the considered microhabitat in the *Sphagnum* environment may affect the response of *Sphagnum* to nitrogen deposition (Heijmans, 2008; Breeuwer, 2009; Utstol-Klein, 2015; Krebs, 2016; van den Elzen, 2017). In addition, a number of studies have found that *Sphagnum* has a threshold for nitrogen deposition saturation. Nitrogen saturation values for *Sphagnum* in Europe have been determined based on greenhouse studies, nitrogen deposition gradient experiments, field experiments on net primary productivity, and investigations of other biogeochemical effects, and the critical nitrogen load for bogs and barren bogs in Europe was considered to be $5\text{--}10 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ and $10\text{--}15 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$, respectively (Bobbink and Hettelingh 2011; Tipping et al. 2013; Wieder R K, 2020). When nitrogen deposition exceeds the critical load required by *Sphagnum*, excess nitrogen will infiltrate the peatland and stimulate vascular plant growth (Wieder, 2019), which may affect carbon accumulation in *Sphagnum* and gradually transform the ecosystem from a carbon sink to a carbon source.

The nitrogen cycle encompasses the migration, transformation and turnover of nitrogen in the Earth's atmosphere, biosphere, soil and hydrosphere, with atmospheric nitrogen deposition being an important part. Climate conditions such as temperature and precipitation vary greatly among latitudes, and the levels of economic, and agricultural development and environmental protection policies differ among countries; therefore, atmospheric nitrogen deposition may vary at different latitudes. Since *Sphagnum* is highly sensitive to nitrogen deposition, we conjecture that the response of *Sphagnum* to nitrogen deposition varies at different latitudes. Previous studies have focused on the *Sphagnum* community in a single region or a single latitudinal zone, and there is a lack of assessments of the spatial heterogeneity of the response of *Sphagnum* to nitrogen deposition on a larger spatial scale. It is still unclear how environmental factors at different latitudes affect the response of *Sphagnum* to nitrogen deposition, so quantitative assessments of such factors are needed. The nitrogen deposition saturation values for *Sphagnum* are lacking, and research on the nitrogen saturation values in different latitudinal zones is even rarer.

Meta-analysis is a method in which the results of a large number of independent studies with a common research purpose are quantitatively combined to analyze the characteristics of the differences among the studies and comprehensively evaluate the results (Ellenberg, 1988). Meta-analysis gives scientific weight to each study, excluding as much bias as possible, and the results are more scientifically valid than other

statistical analysis methods. In this study, we extracted the results of 66 published and unpublished nitrogen deposition studies to analyze the response of *Sphagnum* growth to nitrogen deposition at different latitudes and to quantify the nitrogen saturation values. We divided the data into three zones by latitude: a lower latitudinal zone below 50°, a mid-latitudinal zone at 50–60°, and a higher latitudinal zone above 60°. A mixed-effects model was developed for different latitudes to analyze how environmental factors such as temperature, precipitation, microhabitat, and local nitrogen deposition affect the response of *Sphagnum* to nitrogen deposition. The nitrogen deposition saturation values for *Sphagnum* at different latitudes were determined by linear regression to analyze the differences among latitudinal zones. We explored the response of *Sphagnum* to nitrogen deposition and demonstrated that excessive nitrogen deposition inhibited *Sphagnum* growth. The determined nitrogen deposition saturation values for *Sphagnum* will provide scientific indicators for artificial nitrogen fertilization and for maintaining the carbon storage function of *Sphagnum* plants.

Materials And Methods

Data acquisition

We summarized data from 66 nitrogen experiments with *Sphagnum* in 48 experimental peatland areas in Europe, North America, Asia, and South America (Fig. 1). The published data were mainly obtained from the Web of Science database, with “Sphagnum”, “nitrogen”, and “fertilization” as keywords, and the screening conditions were as follows: (i) The study had to evaluate two or more levels of nitrogen applied to *Sphagnum* and ensure that the temperature, solar radiation, moisture conditions and other nutrients were consistent with those of the control. (ii) Fertilization studies had to have been carried out in the field or indoor culture experiments in which materials were collected outdoors. (iii) The variable reflecting the *Sphagnum* growth must have been production. In addition, this selection of literature was supplemented with a search of Google Scholar, and data from a total of 45 peer-reviewed studies were collected (S3). To avoid publication bias to the greatest extent possible, in addition to the abovementioned published data, a portion of the data was obtained from relevant unpublished experiments shared by *Sphagnum* researchers. All data were used after receiving the author’s permission.

The nitrogen deposition response of *Sphagnum* has been studied the most in North America and Europe, and *Sphagnum* tends to grow in mid- and higher latitudes where the environment is cold and humid. Therefore, when latitude is analyzed as a continuous variable, the significance of areas with more experimental data and widespread distribution of *Sphagnum* is weakened, while the significance of areas with less experimental data and sparse distribution is amplified. This will lead to the final results showing an insignificance of latitude, but we believe that the possibility of regularity with latitude cannot be excluded. We divided the experimental data into three latitudinal zones according to the number of experimental data points and the growth habit of *Sphagnum* to ensure that the amount of data in each latitudinal zone was sufficient for meta-analysis, and the results were convincing.

Variable description

We used the annual production of *Sphagnum* as a response variable; nitrogen application, background nitrogen level, mean annual precipitation, mean annual temperature, mean July temperature (data for the Southern Hemisphere were converted to maximum average summer temperatures), and microhabitat as explanatory variables, of which microhabitat as a categorical variable; and the rest (nitrogen application, background nitrogen level, mean annual precipitation, mean annual temperature, mean July temperature) as continuous variables. The annual production of *Sphagnum* refers to the annual production or *Sphagnum* biomass ($\text{g.m}^{-2}.\text{yr}^{-1}$) and was averaged over several years if the experiment was performed for a few years. Nitrogen application refers to the total amount of nitrogen ($\text{g.m}^{-2}.\text{yr}^{-1}$) applied to the experimental area in one year or the last year of data collection if the experiment was conducted over several years. Background nitrogen is the amount of wet nitrogen deposition ($\text{g.m}^{-2}.\text{yr}^{-1}$) that occurred in the experimental area. When data were not available in the literature, relevant nitrogen deposition data for the study area were obtained from local meteorological websites or from other studies. Annual mean precipitation is the annual mean precipitation (mm.yr^{-1}) within the peat bog in which the experiment was located, or if data were not available for that peat bog, data for the area surrounding the bog were obtained. Temperature variables included annual mean temperature and July mean temperature. The mean temperature in July has been shown to have the highest correlation with the summer mean temperature (Limpens, 2011); therefore, it was used as the summer temperature variable in the study area. There were mainly two types of microhabitats, hummock and hollow (include lawn), and the microhabitat variable was used as a measure of the marsh water table.

Methods

Meta-analysis is the quantitative, scientific synthesis of research results. In a meta-analysis, one or more outcomes in the form of effect sizes are extracted from each study (Jessica, 2018). The effect size represents the degree of difference between the control group and the experimental group and can indicate the changes in *Sphagnum* growth following increased nitrogen deposition. In this study, log response ratios were used as effect sizes in the meta-analysis. The effect size and within-study variance for each study were calculated based on the mean, sample size, and standard deviation of the response variables using the following formula:

$$y_i = \ln R = \ln \frac{x_e}{x_c} \quad (\text{A.1})$$

$$V_i = \frac{S_e^2}{N_e X_e^2} + \frac{S_c^2}{N_c X_c^2} \quad (\text{A.2})$$

Eq. (A.1) is used to calculate the effect size, y_i , and Eq. (A.2) is used to calculate the within-study variance, V_i , where X_e is the mean value of the experimental group, X_c is the mean value of the control group, S_e is the standard deviation of the experimental group, S_c is the standard deviation of the control group, N_e is the sample size of the experimental group, and N_c is the sample size of the control group. A random-

effects model was established in this study, with *Sphagnum* production as the response variable, and the combined effect size was used as the basis upon which to analyze the effect of nitrogen deposition on *Sphagnum* growth. On the basis of the random-effects model, a mixed-effects model was established by introducing explanatory variables to analyze the significance of each factor to the reaction of *Sphagnum* to nitrogen deposition, with the mixed-effects model both taking into account the variation among studies and explaining the large heterogeneity in the random-effects model. Veroniki et al. (2019) stated that restricted maximum likelihood (REML) is the best method for calculating interstudy variance for both dichotomous and continuous data because it yields unbiased parameter estimates, so the REML method was used to calculate the interstudy variance here. To identify the overall reaction of *Sphagnum* to nitrogen deposition across all studies, the combined effect sizes were used, which were weighted as the reciprocal of the within-study variance and the sum of the between-study variances (Ellenberg, 1988). According to the mixed-effects model established to fit the regression function ($Y = ax + b$) to the values of the response variable, the fitted function Y represents the combined effect size of the explanatory variables, and x represents the value of the explanatory variable. When the combined effect size (Y) is 0, the response variables are not affected by the explanatory variables, which means that the critical value of the explanatory variables has been reached, from which we can obtain the nitrogen saturation value required for the *Sphagnum* growth. Since there may be redundancy in the results obtained by including all the significant factors in the model, the listed significant factors were ranked on the basis of the mixed-effects model, all the possible models were analyzed, and the AIC values were compared among models to identify the optimal model. All the above modeling and testing procedures were implemented in R studio version 3.6.1 using the 'metafor', 'ggplot2', 'glmulti' and 'readxl' packages (Viechtbauer, 2010).

Meta-analysis model diagnosis is usually performed with a failsafe number or a funnel plot, both of which can be used to determine the reasonableness and stability of the model. The failsafe number was first proposed by Rosenthal (1979) to assess the degree of publication bias by calculating the minimum number of unpublished studies needed to overturn the conclusions of the meta-analysis. Meta-analysis results are considered reasonable and highly stable when the calculated failsafe number is greater than $5k + 10$ (where k is the number of studies included in the analysis). A funnel plot indicates the accuracy of the model by specifying the symmetry of Egger's regression test for quantitative calculations. When the p -value is greater than 0.05, the model is considered to have high reliability, with a funnel with strong symmetry indicating high reliability of the conclusion (Egger M, 1998).

Results

Reaction of *Sphagnum* to nitrogen deposition at different latitudes

The combined effect sizes for all three latitudinal zones were negative, indicating that nitrogen deposition would inhibit *Sphagnum* production regardless of the latitudinal zone. P -values were greater than 0.05 in all zones except the mid-latitudinal zone, indicating that the results at lower and higher latitudes were not

significant, which is related to the number of experiments. Data for mid-latitudes were sufficient to conclude that the effect of nitrogen was significant, but the conclusions for lower latitudes and higher latitudes need more experimental results to determine the significance of this effect. The absolute value of the combined effect size decreased with increasing latitude, with a combined effect size of -0.1323 in the lower latitudinal zone, -0.0691 in the mid-latitudinal zone and only - 0.0449 in the higher latitudinal zone (Table 1). The absolute value was low at all but the lower latitudes, suggesting that the inhibitory effect of increasing N application on *Sphagnum* growth became less pronounced at higher latitudes, probably due to the lower background N levels in these areas.

Table 1
Meta-analysis of the reaction of *Sphagnum* to N deposition in different latitudinal zones

	Combined effect size	Standard deviation	Upper	Lower	p	τ^2	n
Lower latitude	-0.1323	0.0737	-0.2768	0.0122	0.0728	0.1820	50
Mid-latitude	-0.0691	0.0212	-0.1105	-0.0276	0.0011	0.0308	139
Higher latitude	-0.0449	0.0456	-0.1342	0.0445	0.3248	0.0789	60
Note: Upper and lower represent the boundaries of the 95% credible intervals. τ^2 , residual heterogeneity. n, number of experiments involved in the calculations.							

Analysis of significant factors

Due to the regular differences in the combined effect sizes of *Sphagnum* production at different latitudes, it was necessary to further explore the factors related to the latitudinal reaction of *Sphagnum* to nitrogen deposition. Therefore, we established optimal models for *Sphagnum* production at different latitudes, and the results showed that the related factors also differed among latitudes (Table 2). Nitrogen deposition was found to be a significant factor in the optimal model for all three latitudinal zones, but the roles of the two nitrogen deposition-related factors (nitrogen application, background nitrogen level) differed at the different latitudes. Production at lower and higher latitudes was positively correlated with the background nitrogen level and negatively correlated with the nitrogen application rate, suggesting that the current rate of nitrogen deposition at higher and lower latitudes can enhance *Sphagnum* production, while continued nitrogen application or sustained increases in nitrogen deposition may inhibit production. The combined effect sizes of production for background nitrogen were 0.0306 at lower latitudes and 0.0921 at higher latitudes (Table 2), indicating that background nitrogen application at higher latitudes more strongly promotes *Sphagnum* production. The combined effect size of production for nitrogen application at lower latitudes was the lowest, indicating that sustained increases in nitrogen application have the greatest impact at lower latitudes. The combined effect sizes of production for both nitrogen deposition factors were negative at mid-latitudes, indicating that the current rate of nitrogen deposition inhibited *Sphagnum* growth in the region.

At mid-latitudes, in addition to nitrogen deposition-related factors, microhabitat type was also a significant factor, with combined effect sizes of -0.1211 for production in hummocks and - 0.0453 for production in hollows, indicating that *Sphagnum* plants in hummocks have a stronger inhibitory effect when production is suppressed by nitrogen deposition. Moreover, the significant factors at higher latitudes were precipitation and temperature, both of which had negative combined effect sizes, suggesting that persistently elevated temperatures and rainfall suppress *Sphagnum* production. The p-values for most of the explanatory variables in the model satisfied the t-test and showed significance. The higher latitudes in the three optimal models showed the highest percentage of explanation, reaching 85.69%, while the other latitudes had lower explanatory power.

Table 2
Results of the optimal models of *Sphagnum* production for different latitudes

	Combined effect size	Standard deviation	Lower	Upper	p	τ ²	n
Lower latitudes						18.24%	42
Intercept	-0.0527	0.1864	-0.4181	0.3127	0.7773		
N application	-0.0096	0.0029	-0.0153	-0.0040	0.0009		
Background N	0.0306	0. 0223	-0.0131	0.0743	0.1696		
Mid-latitudes						24.63%	137
Intercept	0.0951	0.0440	0.0089	0.1813	0.0305		
N application	-0.0012	0.0006	-0.0024	-0.0001	0.0612		
Microhabitat	-0.1206	0.0478	-0.2142	-0.0269	0.0116		
Background N	-0.0078	0.0029	-0.0134	-0.0021	0.0068		
Higher latitudes						85.69%	58
Intercept	1.8228	0.4611	0.9191	2.7266	< 0.0001		
N application	-0.0041	0.0011	-0.0064	-0.0019	0.0003		
Annual precipitation	-0.0028	0.0007	-0.0043	-0.0013	0.0002		
Annual temperature	-0.1383	0.0234	-0.1841	-0.0925	< 0.0001		
Background N	0.0921	0.0263	0.0406	0.1437	0.0005		
Note: Upper and lower represent the boundaries of the 95% credible intervals. τ ² , residual heterogeneity. n, number of experiments involved in the calculations.							

Nitrogen saturation value

In this study, total nitrogen is defined as the sum of applied nitrogen and background nitrogen. We calculated two types of nitrogen saturation values. One is the saturation value for nitrogen application: when the amount of applied nitrogen exceeds the saturation value, it will inhibit *Sphagnum* growth. The other is the saturation value for total nitrogen deposition: when the sum of background nitrogen and applied nitrogen exceeds this value, it will inhibit *Sphagnum* growth. The saturation value for nitrogen application can be applied to the agricultural fertilization and industrial cultivation of *Sphagnum*, and the saturation value for total nitrogen deposition can provide some basis for research on the physiological nitrogen requirements of *Sphagnum*. As shown in Table 3 and Fig. 2, the *Sphagnum* nitrogen saturation values were lowest at mid-latitudes, followed by lower latitudes, and were highest at higher latitudes. The p-values corresponding to the 95% confidence intervals of the t-tests for nitrogen saturation indicated significance across all analyses. When the explanatory variable was nitrogen application, both the slope and the intercept were negative in the linear regression function for mid-latitudes, resulting in there being no nitrogen saturation value. The saturation values for nitrogen application were always lower than those for total nitrogen. The difference between them essentially reflects the background nitrogen value in the region, which was higher than the global nitrogen saturation values in all zones except the mid-latitude zone, indirectly indicating the higher background nitrogen level at mid-latitudes.

Table 3
Nitrogen saturation values for *Sphagnum* at different latitudes

Latitudinal zone	Explanatory variable	Fitting function	N saturation (kg ha ⁻² yr ⁻¹)	p
Global	N application	Y=-0.0026x + 0.0272	10.34	< 0.0001
Lower latitudes		Y=-0.0059x + 0.0929	15.75	0.0083
Higher latitudes		Y=-0.0048x + 0.1306	27.21	0.0063
Global	Total N	Y=-0.0029x + 0.0561	19.34	< 0.0001
Lower latitudes		Y=-0.008x + 0.1579	19.74	0.0090
Mid-latitudes		Y=-0.0018x + 0.0180	10	0.0039
Higher latitudes		Y=-0.0047x + 0.1374	29.23	0.0070

Model validation

In this study, the failsafe numbers for the lower- and mid-latitude models were 231 (k = 42) and 1391 (k = 137), respectively, and both models passed the test. The higher-latitude model also passed the test, with a funnel plot p-value greater than 0.05. Thus, the model validation results show that our conclusions are reliable, and the funnel plots for each model are detailed in Appendix 5.

Discussion

Reaction of *Sphagnum* to nitrogen deposition

The total carbon content of peatlands averages $47 \pm 6\%$ (Loisel, 2014), and a large part of this value depends on *Sphagnum* plants. The acidic, nutrient-poor and water-saturated environment in which *Sphagnum* species grow makes it difficult for dead plant tissue to decompose (Hayward and Clymo, 1982). In addition, the polyphenol metabolites produced by *Sphagnum* plants have antimicrobial properties and inhibit microbial decomposition (Jassey, 2013). Therefore, changes in *Sphagnum* biomass will greatly affect carbon fixation in peatlands. The results of this study indicate that excessive nitrogen deposition will inhibit *Sphagnum* growth (Table 2), whereas nitrogen deposition not exceeding the saturation values can promote *Sphagnum* growth (Table 3). A meta-analysis by Limpens et al. (2011) also showed that low nitrogen application in areas with low nitrogen deposition promotes *Sphagnum* production, while high nitrogen application inhibits *Sphagnum* production. The effects of changes in nitrogen deposition on *Sphagnum* growth may further affect carbon sequestration. We conclude that when nitrogen deposition promotes *Sphagnum* growth, this increase in production will slow the decomposition of litter in the environment, thus enhancing carbon sequestration. Conversely, when nitrogen deposition exceeds the saturation value, the effect on carbon sequestration will be reduced.

The inhibitory effect of increasing total nitrogen deposition on *Sphagnum* production became less pronounced at higher latitudes, which may be related to human activities. The data from lower latitudes included in this study were mainly obtained from eastern Asia, with rapid development occurring at latitudes below 50° , where industrial development over the last two to three decades has resulted in the production of large amounts of nitrogen oxides. Together with the long-term application of nitrogen fertilizers in many places, this has led to the occurrence of excessive nitrogen in adjacent peatlands, such as those in southwestern and northeastern China (Li TT, 2018; Du EZ, 2014). It has been shown that nitrogen deposition in East Asia has increased by $0.6 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-2}$ over the last 20 years; however, nitrogen deposition peaked in 2010–2012 and is expected to decrease in the future (Geddes, 2017). We suggest that *Sphagnum* at lower latitudes is currently affected most by nitrogen deposition and that when the environment is managed according to the saturation values identified in our study, this effect will diminish, and the carbon sequestration capacity of *Sphagnum* will eventually recover.

Compared with that in the lower latitudinal zone, the *Sphagnum* in the mid-latitudinal zone has a relatively lower impact on nitrogen deposition. A study by Fritz et al. (2014) showed that the rate of nitrogen uptake by *Sphagnum* decreases sharply under prolonged nitrogen stress, which means that high nitrogen uptake may evolve in *Sphagnum* over the long term. The mid-latitudinal regions in this study mainly occur in Europe and North America, which have experienced a long industrial revolution and where the rate of nitrogen deposition is generally high. Furthermore, nitrogen deposition in North America and Europe has shown an increasing and then decreasing trend over the last century, and Geddes et al. (2017) showed that nitrogen deposition decreased by $0.6 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-2}$ in North America and $0.1 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-2}$ in Europe over the last 20 years. It has been shown that *Sphagnum* detoxification of excess nitrogen is slow and requires both energy and products of photosynthesis production. Therefore, a mechanism for the

downregulation of nitrogen uptake has evolved in *Sphagnum* species to prevent excessive uptake of nitrogen and energy loss (Heeschen, 1996; Chiwa, 2018). To adapt to a long-term existence in a high-nitrogen-deposition environment, *Sphagnum* plants will reduce the rate of nitrogen uptake and thus suppress *Sphagnum* production (Fritz, 2014). Adaptation to high nitrogen loads may occur over several years or decades of increased nitrogen deposition. Although nitrogen deposition may decrease due to legislation, this adaptation to high nitrogen will be maintained long after the decrease (Sally, 2006; Philippine, 2008). Nitrogen deposition at mid-latitudes peaked earlier and has been declining for more than 10 years, and the adaptation to high nitrogen gradually disappeared. However, nitrogen deposition at lower latitudes has also reached its peak, but a strong adaptation to the high-nitrogen-deposition environment may still be maintained. Thus, *Sphagnum* plants at lower latitudes are more significantly inhibited by excess N than are those at mid-latitudes. We propose that the adaptation of *Sphagnum* to high nitrogen at lower latitudes will gradually disappear with reasonable artificial intervention to reduce nitrogen deposition. This will benefit *Sphagnum* growth and facilitate carbon sequestration by *Sphagnum* species.

High latitudes are less inhabited than lower latitudes and remain relatively pristine, with less nitrogen deposition. Furthermore, the productivity of the entire ecosystem at these latitudes is limited by nutrient deficiencies and climatic conditions such as low and freezing temperatures (Diakova, 2016), making such regions less affected by the effects of increasing nitrogen deposition on the carbon sequestration capacity of *Sphagnum* plants than lower-latitude regions.

Significant factors and future trends

The significant factors varied by latitude, and the nitrogen deposition-related factors were all found to be extremely significant, indicating that nitrogen deposition has a significant effect on *Sphagnum* growth (Fig. 3.a). In the mid-latitudinal zone, the microhabitat type was also found to be significantly related to *Sphagnum* growth, which was not evident at lower or higher latitudes (Fig. 3.b). This may be related to the significant differences in the retention of nitrogen in different water tables among hummocks, hollows at mid-latitudes. Since *Sphagnum* production is highly correlated with water table fluctuations in the microhabitats in which the plants occur, a relative decrease in water level will result in a significant decrease in production (Gaudig, 2020). Higher-latitude areas have the most significant factors. In addition to nitrogen deposition, *Sphagnum* production is closely related to precipitation and temperature in this region due to the low level of human activity and is extremely sensitive to environmental changes (Fig. 3.c).

In recent decades, there has been an increasing global temperature trend, with a particularly pronounced increase in minimum temperatures (Rosmann, 2016). We found that higher temperatures at higher latitudes will inhibit *Sphagnum* growth (Fig. 4). Mineralization of peat would likely increase with warming, while greater evaporation at higher temperature leads to a lower water table, which will promote the growth of vascular plants in peatlands, thus increasing shading and eventually suppressing *Sphagnum* growth (Richard, 2019). A sustained increase in temperature may cause the respiration rate of *Sphagnum* plants to surpass their photosynthetic rate, leading to a reduction in the synthesis and storage of organic

matter and then a weakening of the role of *Sphagnum* in sequestering carbon in peatlands. With global warming, the annual precipitation range has also increased at mid- to high latitudes (Fig. 4), and the rate of increase in maximum precipitation has exceeded the rate of increase in minimum precipitation (Chou chia, 2012). Our study shows that increased precipitation at higher latitudes will inhibit *Sphagnum* growth. The average residence time of water in the *Sphagnum* layer after low-intensity precipitation is 10–30 min, but excessive rainfall > 5 mm may remain for only 0.5–5 min in the uppermost *Sphagnum* layer (Martina, 2005; Holden J, 2009). Therefore, the higher frequency of extreme precipitation conditions at higher latitudes over the last half century may have resulted in obvious nitrogen leaching, leading to the weakened metabolic activity and growth of *Sphagnum* plants. Furthermore, the intensification of extreme precipitation and extreme cold conditions has been underestimated globally over the last fifty to sixty years (Fischer EM, 2014). The combination of these extremes with continued warming and increased precipitation will result in rapid and deep infiltration of rainwater and solutes (Holden J, 2009; Jeremy A, 2016; McCarter, 2020), which will significantly impact *Sphagnum* growth and further affect the ability of peatlands to sequester carbon.

Saturation value

Our study shows that there is no saturation value for nitrogen application at mid-latitudes, which indicates that the current nitrogen application rate has already exceeded the nitrogen saturation value required for *Sphagnum* growth. The total nitrogen saturation values at mid-latitudes are also far below those at lower and higher latitudes (Fig. 5). Thus, a continued increase in nitrogen deposition would inhibit *Sphagnum* growth. Fortunately, the current mid-latitude nitrogen deposition rate is gradually decreasing. However, the results are also caused by the fact that the distribution of the data in this study is mainly concentrated in Europe, and data from more regions should be incorporated into future analyses. Moreover, the nitrogen saturation values were higher at higher latitudes. In cold, nitrogen-stressed ecosystems, *Sphagnum* plants may derive a large amount of their nitrogen requirement from free amino acids, with a different nitrogen requirement compared to that in other regions (Krab EJ, 2009). It is possible that *Sphagnum* plants are influenced by the microclimate at high latitudes (Krab EJ, 2013). *Sphagnum* plants also associate with N-fixing microbes that could provide nitrogen to their moss hosts (Carella P, 2018; W.K. Sexton, 2020), which needs to be explored further in future studies.

Differences in nitrogen saturation values at different latitudes can be a good indicator of peatland ecological restoration, especially in mid- and lower-latitude areas, which have high nitrogen deposition rates and high levels of human activities. Attention should be paid to agricultural fertilization, in addition to controlling nitrogen oxide emissions. According to the local nitrogen sedimentation background, scientifically calculating the nitrogen application rate can assist in the development of reasonable fertilization measures, not only providing a basis for ecological protection but also facilitating carbon sequestration in ecosystems by *Sphagnum* species.

Conclusion

The results show that excessive nitrogen deposition inhibits *Sphagnum* growth and that the inhibitory effect of increasing nitrogen application on *Sphagnum* growth decreases with increasing latitude. The nitrogen saturation value for *Sphagnum* varies with latitude, with the lowest value found at mid-latitudes. This indicates that mid-latitude areas are the most heavily influenced by anthropogenic activities and that *Sphagnum*-containing ecosystems are approaching nitrogen saturation. The reasonable use of nitrogen saturation values for different latitudes can provide a theoretical basis for precision fertilization in agriculture and peatland protection.

Significant factors affecting the *Sphagnum* response to nitrogen deposition differ with latitude, with the exception of nitrogen deposition; microhabitat type is a major factor affecting *Sphagnum* growth at lower latitudes, with microhabitats possessing a higher water table being favorable to *Sphagnum* growth. Precipitation and temperature are also important to *Sphagnum* growth at higher latitudes, and a continuous increase in temperature and precipitation is detrimental to *Sphagnum* growth. Based on the significant nitrogen deposition-related factors affecting *Sphagnum* growth, a reasonable protocol can be specified for *Sphagnum* culture and further applied in commercial production.

Overall, the interpretation of the model of the *Sphagnum* reaction to nitrogen deposition remains inadequate due to the presence of unconsidered factors such as sunlight, symbiotic plants and the frequency of extreme weather. The light factor will affect *Sphagnum* photosynthesis, which directly relates to its growth and production. Symbiotic plants will impact the shading of *Sphagnum* plants, which will further relate to its light condition, and the nutrient uptake by symbiotic plants will affect the nutrient absorption by *Sphagnum* plants. Extreme weather, such as drought and freezing, will change the temperature and moisture conditions, which will further relate to the *Sphagnum* nitrogen uptake and growth conditions. Furthermore, we did not discuss interactions between factors, such as the interaction between temperature and precipitation and that between microhabitat and precipitation. To improve the explanatory ability of the model, subsequent studies could discuss the interactions between climatic and geographical factors. The spatial correlations between significant factors should be further investigated by collecting data from additional areas, which will help to improve the understanding of spatial differences in *Sphagnum* characteristics and of *Sphagnum*-related mechanisms and facilitate applications of the nitrogen saturation values. The development of high-resolution remote sensing satellite imaging and unmanned aerial vehicle (UAV) technology in recent years has provided the possibility to perform this aspect of research.

Declarations

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Figures

Figure 1

Distribution of samples collected in Sphagnum nitrogen application experiments Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

(a), (b), (c), and (d) show the global, lower-latitude, mid-latitude, and higher-latitude Sphagnum combined effect sizes associated with nitrogen deposition and saturation. The dashed red lines represent an effect size of 0, the dashed black lines represent the upper and lower boundaries of the confidence interval, the solid black lines represent the relationship between the effect size and nitrogen application, and the dashed blue lines represent the saturation value. Each gray dot represents a nitrogen application study, with larger dots indicating greater weight.

(a) **Model-averaged importance of terms**

(b) **Model-averaged importance of terms**

(c) **Model-averaged importance of terms**

Figure 3

Importance of significant factors at different latitudes. (a) Significant lower-latitude explanatory variables, (b) significant mid-latitude explanatory variables, and (c) significant higher-latitude explanatory variables. The x axis indicates the level of importance, and values above 0.8 are considered highly important.

Figure 4

Higher-latitude Sphagnum effect sizes were associated with annual temperature and annual precipitation. The dashed red lines represent an effect size of 0, the dashed black lines represent the upper and lower boundaries of the confidence interval, and the solid black lines represent the relationship between the effect size and annual temperature or annual precipitation. Each gray dot represents a nitrogen application study, with larger dots indicating greater weight.

Figure 5

Nitrogen saturation values of Sphagnum at different latitudes

Supplementary Files

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