Supporting information

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Title: Chronic atmospheric reactive nitrogen deposition suppresses biological nitrogen fixation in peatlands.

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Material and methods supplementary information

Checks on $^{15}N_2$ gas for contamination. The $^{15}N_2$ gas used for the incubations can be contaminated with readily available forms of ^{15}N such as $^{15}NH_3$ or $^{15}NO_x$ (nitrate/nitrite),[29] which will result in erroneous figures of BNF rates. To avoid this problem, we tested the $^{15}N_2$ gas (98 atom% Cambridge Isotope Laboratories Inc., USA) and we found some contamination that consisted of a difference in average results of enriched and non-enriched samples of δ ^{15}N ‰ of 0.03. Thus, this figure was used as a threshold below which no BNF was reported.

Elemental analyses in *Sphagnum* tissue and peat. A set of subsamples of pulverized *Sphagnum* tissue and peat from each of the species of each site were analysed for total C and N content. They were sent to the laboratory of the School of Geographical Sciences at the University of Bristol. They were analysed using a Thermo Scientific Flash EA 1112 Nitrogen and Carbon analyser. The instrument had a limit of detection (LOD) for both C and N of 0.01%, and the precision was determined by repeated analysis of a soil reference standard (0.21% N and 2.39% C) and the relative standard deviation (RSD) was below 5%.

Of the ground samples 0.2 grams were digested in 9 ml of HNO_3 (>68%) trace metal grade and 1 ml of H_2O_2 (30%) ACS grade using a microwave Mars 6 CEM (Mathews, NC, USA). The digests were diluted using deionised water and analysed for total P and metals (Mg, K, Ca, V, Mn, Co, Ni, Cu, Mo) using inductively coupled plasma – mass spectrometry (ICP-MS, Perkin Elmer NexION 300D, Waltham, MA, USA). An 8-point calibration generated through dilution of a certified ARISTAR multi-element standard solution for ICP (VWR, UK) was used to determine the values. Additionally, every 9 samples a blank and an external standard sample were included. The results were blank corrected. On average, the RSD was below 4% for all the elements while the LOD was 0.3 μg/g for Mg, K, Ca, Ni, Mo, and P; and 0.1 μg/g for V,

Mn, Co, and Cu.

Ancillary measurements in the field. After each incubation in the field we recorded mean air temperature (ºC) for the sites on that date from stations nearby and measured the temperature in the moss and peat (5-10 cm depth) at the exact location where the samples were taken and incubated using a stem thermometer probe (Premier Farnell Ltd, UK). Additionally, at the exact same spots we measured dissolved oxygen (DO, mg/l) in surface porewater using a portable DO meter (HACH HQ40d with LDO probe, Loveland, CO, USA). pH using a pH meter (HI-98100 Hanna Instruments, Leighton Buzzard, UK). Electrical conductivity (EC, µS/cm) using an EC meter (HI-98300 Hanna Instruments, Leighton Buzzard, UK). And soil moisture (% vol) focused on capturing the moisture of the moss carpet (5-10 cm upper part) and peat layer (5-10 cm depth from the beginning of peat in hollows and hummocks) using a moisture meter type HH2 (Delta-T Devices, Burwell, UK).

Pore water samples were also taken from the incubation locations at each site. The samples were transported to the laboratory immediately after collection in a cool box with ice packs. Then they were filtered through a Restek 0.45µm PTFE syringe filter, 25mm diameter. The filtrates were analysed for nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulphate (SO_4^{2-}) concentration using an ion chromatograph (DIONEX ICS-1000, Sunnyvale, CA, USA). The limits of detection were 0.1 mg L^{-1} for NO_3^-, <0.001 mg L^{-1} for $PO^3_4^-$, and 0.2 mg L^{-1} for SO_4^-. The results were blank corrected and the precision as RSD was <5%. Also, the samples were analysed for ammonium (NH_4^+) using a flow injector analyser (Lachat QuikChem 8500, Hach, Loveland, CO, USA). The limit of detection for $NH_4^+_3$ was 0.07 mg N L^{-1}, the results were blank corrected, and the RSD was <5%.

Peat samples from hollows and hummocks (10 g) were extracted with 50 ml of deionised water for the determination of nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulphate (SO_4^{2-}); and with 50 ml

of 2 M KCl for the determination of ammonium (NH_4^+). The peat slurries were shaken in an automatic shaker for 1 hour at 200 rpm, and subsequently centrifuged at 4000 rpm for 30 minutes followed by a double filtration in which every three samples a blank (deionised water) was included, first through a number 42 Whatman filter paper, and second through a 0.45 µm PTFE Restek 25 mm diameter syringe filter. The analysis was performed as indicated above for the pore water.

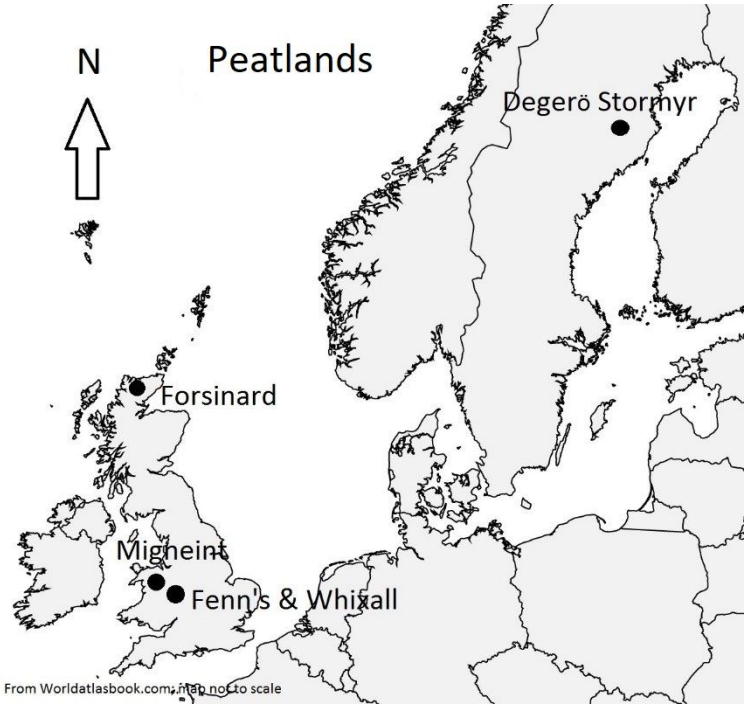

Figure S1. Location of the sampling sites in the UK and Sweden.

Table S1. Description of the treatments of the experimental plots at Degerö Stormyr.

Plot	Treatment	Description
0	None	Control of the experiment, no sampling (undisturbed).
1	n s	Low N and S: 15 and 10 kg ha^{-1} y^{-1} respectively.
2	N S t	High N and S: 30 and 20 kg ha^{-1} y^{-1} respectively, plus greenhouse.
3	S	High S: 20 kg ha^{-1} y^{-1}.
4	t	Greenhouse.
5	S t	High S: 20 kg ha^{-1} y^{-1}, plus greenhouse.
6	n s	Low N and S: 15 and 10 kg ha^{-1} y^{-1} respectively.
7	S	High S: 20 kg ha^{-1} y^{-1}.
8	N S	High N and S: 30 and 20 kg ha^{-1} y^{-1} respectively.
9	N t	High N: 30 kg ha^{-1} y^{-1}, plus greenhouse.
10	S t	High S: 20 kg ha^{-1} y^{-1}, plus greenhouse.
11	Control	No treatment, just mire water added.
12	N	High N: 30 kg ha^{-1} y^{-1}.
13	N S t	High N and S: 30 and 20 kg ha^{-1} y^{-1} respectively, plus greenhouse.
14	N S	High N and S: 30 and 20 kg ha^{-1} y^{-1} respectively.
15	n s	Low N and S: 15 and 10 kg ha^{-1} y^{-1} respectively.
16	t	Greenhouse.

17	N t	High N: 30 kg ha^{-1} y^{-1}, plus greenhouse.
18	N	High N: 30 kg ha^{-1} y^{-1}.
19	Control	No treatment, just mire water added.
20	n s	Low N and S: 15 and 10 kg ha^{-1} y^{-1} respectively.

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101 Table S2. Total reactive nitrogen deposition (Nr; Kg N ha^{-1} yr^{-1}).* Nitrogen deposition by its
102 two major forms: NHx as reduced and NOy as oxidized (in kilo-equivalents per hectare per
103 year: Keq ha^{-1} yr^{-1} - 1 keq N ha^{-1} yr^{-1} is equal to 14 kg N ha^{-1} yr^{-1}). Ratio between the two types
104 of Nr forms. Percentage of each form that comprises the total Nr deposition. Median $\delta^{15}N$
105 values of all studied species for each of the sites.

	Kg N ha^{-1} yr^{-1}	Keq ha^{-1} yr^{-1}			Ratio	%	%	‰
	Total Nr_{dep}	**Total**	**NHx**	**NOy**	**NHx:NOy**	**NHx**	**NOy**	**$\delta^{15}N$**
Fenn's&Whixall	27	1.9	1.66	0.24	6.9	87	13	-5.73
Migneint	17	1.2	0.77	0.41	1.9	65	35	-3.14
Forsinard	6	0.4	0.23	0.17	1.4	58	43	-1.49
Degerö	2	0.1	0.07	0.07	1.1	52	48	-2.26

*****Note that the Nrdep data for the British sites is for the period 2013-2015 and the data for Degerö (Sweden) is for 2014-2016.

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Table S3. Environmental variables for pore water and peat measured during the sampling campaigns (UK sites in June, Swedish site in July).

Abiotic factors	**Units**	**Fenn's & Whixall** Median (±MAD)	**Migneint** Median (±MAD)	**Forsinard** Median (±MAD)	**Degerö** Median (±MAD)
Moss/peat Temperature	°C	**12.4** (±0.9) a	**12.9** (±1.1) b	**10.2** (±0.2) c	**17.1** (±2.4) d
Dissolved Oxygen	mg/L	**4.1** (±2.3) a	**5.7** (±1.0) a	**7.0** (±0.4) b	**ND**
pH		**3.8** (±0.2) a	**4.6** (±0.1) b	**5.0** (±0.2) c	**3.8** (±0.1) a
Electrical Conductivity	µS/cm	**94.5** (±10.0) a	**30.0** (±9.0) b	**38.0** (±1.5) c	**45.0** (±11.0) b
Sphagnum moisture volumetric	% vol	**65.0** (±25.6) a	**46.9** (±34.4) a	**76.9** (±16.1) a	**64.8** (±33.7) a
Sphagnum moisture gravimetric	g/g	**20.7** (±8.7) a	**17.4** (±4.8) a	**17.4** (±6.82) a	**16.0** (±7.9) a
Pore Water NO_3^-	mg/L	**0.256** (±0.002) a	**0.101** (±0.008) b	**0.167** (±0.011) a	**0.112** (±0.008) b
Pore Water NH_4^+	mg/L	**0.114** (±0.024) ac	**0.092** (±0.021) a	**0.059** (±0.005) c	**0.123** (±0.038) c
Pore Water PO_4	mg/L	**0.279** (±0.050) a	**0.333** (±0.102) a	**0.328** (±0.004) a	**0.242** (±0.013) a
Pore Water SO_4	mg/L	**0.151** (±0.030) a	**0.453** (±0.081) b	**0.537** (±0.084) b	**0.135** (±0.058) a
Peat NO_3^-	µg/g	**1.200** (±NA) a	**1.013** (±0.389) a	**1.003** (±0.01) a	**1.280** (±0.051) a

Peat NH_4^+	µg/g	**3.865** (±1.748) a	**6.133** (±5.851) a	**0.090** (±0.044) b	**2.310** (±1.820) a
Peat PO_4	µg/g	**1.735** (±0.632) a	**10.366** (±0.639) b	**5.002** (±0.513) bc	**3.119** (±0.907) ac
Peat SO_4	µg/g	**4.881** (±3.831) ab	**3.876** (±1.463) a	**2.268** (±0.471) b	**1.669** (±1.039) b

Data shown is median (±MAD) per site for years 2016 and 2017 (except Forsinard: only 2017) (n=36 except for peat data n=12. Forsinard half these values). Sites with different letters have significantly different values.

Table S4. Elements in *Sphagnum* mosses.

	Fenn's&Whixall	**Migneint**	**Forsinard**	**Degerö**
Elements	µg/g (±MAD)	µg/g (±MAD)	µg/g (±MAD)	µg/g (±MAD)
Mg	**512.86** (±136.23) a	**1111.68** (±143.27) b	**1238.57** (±101.75) b	**383.20** (±103.09) a
K	**4283.18** (±1243.21) a	**2497.15** (±920.92) b	**2344.12** (±771.34) b	**3071.33** (±522.60) ab
Ca	**305.29** (±90.02) a	**428.71** (±387.29) a	**428.09** (±132.73) a	**251.39** (±161.13) a
V	**79.37** (±0.79) a	**78.41** (±1.26) b	**73.90** (±4.17) b	**74.27** (±0.48) b
Mn	**83.25** (±18.08) a	**79.80** (±30.94) a	**31.83** (±4.32) a	**41.47** (±41.47) a
Co	**19.42** (±0.31) a	**19.61** (±0.70) ab	**16.46** (±1.83) bc	**13.17** (±0.11) c
Ni	**296.10** (±1.67) a	**293.27** (±4.15) b	**246.73** (±1.46) bc	**244.84** (±1.21) c
Cu	**55.88** (±0.52) a	**55.68** (±0.97) a	**48.08** (±0.98) a	**<LOD**
Mo	**331.39** (±1.96) a	**331.34** (±8.08) ab	**287.07** (±4.33) b	**286.34** (±1.49) b
	mg/g (±MAD)	mg/g (±MAD)	mg/g (±MAD)	mg/g (±MAD)
C	**441.32** (±4.16) a	**435.90** (±4.46) b	**442.13** (±5.62) ab	**441.03** (±2.52) a
N	**5.57** (±0.68) a	**5.48** (±0.55) a	**6.88** (±1.64) a	**5.20** (±0.60) a
P	**0.41** (±0.03) a	**0.34** (±0.05) b	**0.34** (±0.12) b	**0.29** (±0.02) c
Ratios				
C:N	**79.68** (±11.46) a	**79.91** (±9.06) a	**65.24** (±14.05) a	**85.79** (±9.63) a
C:P	**1046.29** (±74.30) a	**1333.27** (±188.40) bc	**1265.01** (±646.57) b	**1537.57** (±85.49) c
N:P	**11.99** (±0.73) a	**16.39** (±1.43) a	**20.75** (±3.83) a	**17.00** (±1.69) a

Data shown is median (±MAD) per site (n=12). Sites with different letters are significantly different.

Table S5. Elements in peat.

	Fenn's&Whixall	**Migneint**	**Forsinard**	**Degero**
Elements	µg/g (±MAD)	µg/g (±MAD)	µg/g (±MAD)	µg/g (±MAD)
Mg	**480.21** (±33.05) a	**335.31** (±26.44) b	**1044.35** (±35.43) a	**314.41** (±85.16) b
K	**<LOD**	**33.91** (±12.45) a	**61.14** (±9.67) ab	**165.98** (±21.48) b
Ca	**922.40** (±42.29) a	**139.53** (±63.14) b	**197.76** (±57.11) bc	**413.19** (±149.03) ac
V	**114.90** (±1.88) a	**79.58** (±1.01) b	**69.63** (±0.94) b	**74.59** (±0.81) b
Mn	**<LOD**	**<LOD**	**<LOD**	**<LOD**
Co	**27.53** (±0.80) a	**21.75** (±0.39) a	**17.84** (±0.14) ab	**13.05** (±0.17) b
Ni	**417.10** (±5.99) a	**291.85** (±4.95) ab	**244.18** (±0.10) ab	**239.46** (±5.51) b
Cu	**289.80** (±75.85) a	**172.95** (±28.40) b	**113.25** (±29.20) b	**16.69** (±10.94) b
Mo	**465.02** (±6.79) a	**326.94** (±4.02) ab	**283.11** (±8.33) ab	**281.37** (±4.17) b
	mg/g (±MAD)	mg/g (±MAD)	mg/g (±MAD)	mg/g (±MAD)
C	**512.26** (±1.38) ac	**465.36** (±0.77) b	**533.33** (±1.23) c	**481.21** (±1.82) ab
N	**12.23** (±0.02) ab	**17.00** (±0.07) c	**15.79** (±0.02) bc	**10.76** (±1.77) a
P	**0.29** (±0.01) a	**0.77** (±0.02) b	**0.29** (±0.00) a	**0.54** (±0.13) ab
Ratios				
C:N	**41.80** (±0.03) a	**27.37** (±0.16) b	**33.83** (±0.14) ab	**45.44** (±7.62) a
C:P	**1745.04** (±18.31) ab	**607.77** (±9.18) c	**1852.65** (±16.86) a	**951.04** (±240.10) bc
N:P	**40.30** (±0.33) ab	**22.20** (±0.46) b	**54.93** (±0.07) a	**21.96** (±8.72) b

Data shown is median (±MAD) per site (n=6). Sites with different letters are significantly different.