UK Centre for Ecology & Hydrology

Vegetation analysis and bioindicator checks at Ballynahone Bog SAC

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1. Introduction

Ballynahone Bog is designated as a Special Area of Conservation (SAC) and Area of Special Scientific Interest (ASSI). It is one of the largest intact active raised bogs in Northern Ireland with hummock and hollow pool complexes. The peatland flora includes bog-rosemary *Andromeda polifolia*, and the bog-mosses *Sphagnum fuscum*, *S. imbricatum* and *S. pulchrum*.

Since September 2014, atmospheric NH_3 concentrations have been monitored at eight sites using the UKCEH ALPHA (Adapted Low-cost Passive High Absorption) samplers (Stephens and Tang 2019); see Figure 1 for the sampler locations. This ongoing study shows that, even at the background site 8, furthest away from local sources, the NH_3 concentrations are well above the annual critical level (CLe) of 1 µg $NH_3 m^{-3}$ for lichens, bryophytes and sensitive habitats like peat bogs. Almost everywhere across the bog, even at the background site, the concentrations for most months are above the annual CLe of 3 µg $NH_3 m^{-3}$ for higher plants. Figure 2 shows the annual mean NH_3 concentration at all eight monitoring sites while more detailed information can be found in Appendix IV.

Figure 1: Overview from the ALPHA sampler locations at Ballynahone Bog. The red rectangles to the south-west of the site represent farm 1. From Tang et al. (2018).

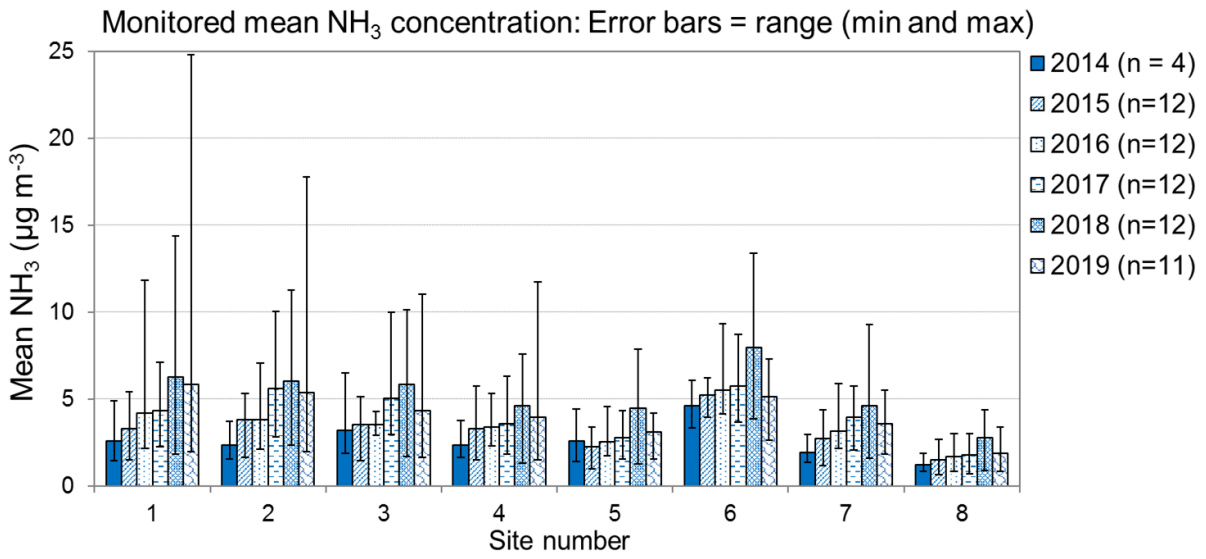

Figure 2: Annual mean NH_3 concentrations (µg m^{-3}) at the eight ALPHA monitoring sites at Ballynahone Bog, with an indication of the minimum and maximum concentration from 2014-2019.

This raised the question of how the vegetation at Ballynahone Bog was affected by the high atmospheric NH_3 concentrations and if there were methods available to show this, even before the damage to the vegetation was visible. Previous biomonitoring studies showed that differences in N content (%N) and foliar ammonium (foliar NH_4-N) in bryophytes were closely related to atmospheric NH_3 concentrations, when there was a point NH_3 source (e.g., a farm) that emitted the gas (Leith at al 2005 JNCC report 386). Of the two indicators, foliar NH_4-N responds more strongly and probably more quickly to NH_3 changes than %N. The report also showed that different plant species react differently to changes in NH_3 air concentration.

A comparison between dry NH_3, wet NO_3-N (nitrate) and NH_4-N (ammonium) inputs at the long-term Whim Bog field manipulation site (Southern Scotland) http://www.whimbog.ceh.ac.uk/ shows that these different forms of N input do not all have the same impact on %N and foliar NH_4-N concentrations of mosses. For %N, the sensitivity appears to be highest for NH_3, intermediate for wet NH_4-N and lowest for NO_3-N. This differentiation is similar but even stronger for foliar NH_4-N in mosses, which were very sensitive to NH_3, but only responded to high levels of wet N deposition inputs (Leith et al 2005 JNCC report 386).

A study into the effects on vegetation at Ballynahone Bog was carried out. This involved two stages; a day visit to the site to assess the visible effects (autumn 2018) followed by a second visit to collect vegetation samples (spring 2019) to quantify the biomonitoring indicators %N and foliar NH_4-N content. This study has used the existing Site Condition Assessments carried out in 2005 and 2011 (NIEA Common Standard Monitoring material), to help to decide on the selection of sampling locations for the N content and foliar NH_4-N analyses.

2. Methods

2.1. Initial visit 21 September 2018

Present: Mark Sutton, Netty van Dijk (both UKCEH (UK Centre for Ecology and Hydrology) Edinburgh), Áine O'Reilly, Kieran McCavana, Sara McGuckin, Paul Corbett, Keith Finegan, James Warnock (all NIEA), Trish Fox (Ulster Wildlife).

Most of Ballynahone Bog is under DAERA ownership with some areas of the site under private ownership. The bog is managed by Ulster Wildlife. DAERA (formerly DOE) acquired the site just before large-scale peat cutting operations were due to commence with drains already put in place. Since then, Ulster Wildlife has undertaken drain blocking works, at critical places, to restore the high-water table. The site is part of the Collaborative Action for Nature Network (CANN) project supported by the EU's INTERREG VA programme, which aims to identify and deliver the conservation measures of these sites to ensure their long-term sustainability.

During this first visit, a walk-over survey was undertaken to assess the vegetation, in preparation for the sampling to be undertaken in spring 2019. Spring is the best time to collect vegetation samples for the %N and NH_4-N analysis, as at this time of year the N content is not yet diluted due to new plant growth. Using samples collected at other times of the year will give different results. The latest Site Condition Assessment data was also used (2011) to guide the location of biomonitoring sampling, but it is acknowledged that the situation might have changed since the last assessment in 2011.

The Site Condition Assessment is designed to give an overview of the condition (favourable or otherwise) of the vegetation, caused by several factors. Condition assessments can highlight that a damaging change is occurring to a site/habitat. While specific pressures and threats can be inferred, it is not a simple cause and effect model and requires further evidence to be considered, including further analysis such as this. When looking for damage caused by a certain factor, in our case NH_3, the addition of sensitive key species to the Common Standards Monitoring methodology would be useful. This would of course be more time consuming.

2.2. Biomonitoring

2.2.1 Sample locations

To enable robust interpretation of the results it was important to collect vegetation samples through the whole available range, from plants expected to be damaged, to healthy plants. As different species react differently to NH_3 we sampled from different sensitive plant groups: bryophytes, *Sphagnum* mosses and lichens. To remain within agreed timeframes and budgets for the study, we restricted the number of samples to 50, which provided a sufficient number of samples across the site. The following criteria were used to decide on the sampling locations:

- **Relate damage of the vegetation to NH_3 air concentrations:** Choose vegetation sampling points as close as possible to the eight existing NH_3 concentration monitoring sites (labelled S1-S8 in Figure 1). S1 is on the boundary of the bog, as close as possible to farm 1.

- **Include sampling locations where no/least damage is expected as reference sites:** Site 8 of the existing NH_3 air sampling locations is the furthest away from known local sources, and S5 is furthest away along the direction of the transect away from farm 1. Therefore, S5 and S8 are the locations where the least damage is expected. Another cleaner location (P35) was added as this was far away from a) the local sources, b) the edges of the bog, c) an area contaminated by lead, and d) was in favourable condition according to the 2011 Site Condition Assessment.
- **Gap in tree line**: samples were taken parallel to and as close as possible to the boundary fence of the bog. (V numbers; note V2 and S6 are in the same location). There are areas along the edge of the bog with bog woodland which may be providing some shelter and recapture of atmospheric N before plumes reach the open bog. The vegetation sampling took account of both the tree shelter and lack thereof along a transect.
- **Farm 2**: Additional sampling locations on the bog were selected close to farm 2. B2 was in favourable condition and C2 was in undecided condition according to the 2011 Site Condition Assessment.

An overview of vegetation sampling locations can be found in Figure 3.

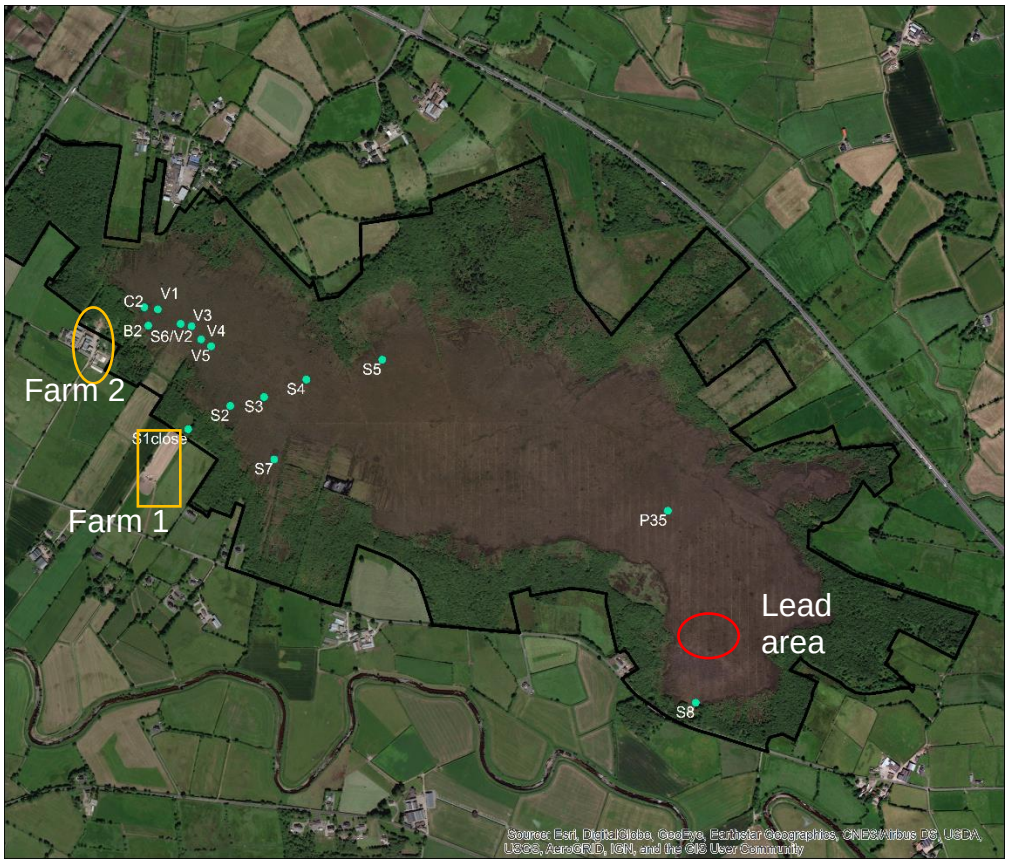

Figure 3: Overview of vegetation sample locations at Ballynahone Bog (March 2019).
The sites labelled S1-S8 are the locations of NH_3 concentration monitoring, whereas the other locations are linked with the Site Condition Monitoring sites. S5, S8 and P35 are the 'reference sites', which are the furthest away from known NH_3 sources.

2.2.2 Sample collection and processing

Samples of the following species were collected on 19th and 20th March 2019 by Netty van Dijk (UKCEH) and Áine O'Reilly (NIEA): *Sphagnum capillifolium*, *Sphagnum subnitens*, *Hypnum*, *Cladonia portentosa*, and *Pleurozium schreberi*. The methodology requires the collection of healthy-looking, intact vegetation samples of the same species at all sample locations. In practice there was not always healthy-looking vegetation present at each sample location, but samples were still collected in these cases. For each sample, a note was made about the healthiness of the vegetation.

All samples were processed and analysed for %N (DW, dry weight) and foliar NH_4-N (µg g^{-1} FW, fresh weight) following the detailed protocols in Appendix II (Van Dijk et al 2009). After studying the results for the foliar NH_4-N (µg g^{-1}FW, fresh weight) it was noticed that there was quite a variation between the samples. In an attempt to limit this variation, we estimated the % dry weight of the vegetation samples and applied this to the calculations for foliar NH_4-N; for details see Appendix II.
This dry NH_4-N (µg g^{-1}DW, dry weight) might also be better comparable with %N, which is also expressed as DW.

3. Results and Discussion

3.1. Initial visit and observations

Near air monitoring site S8, the cleanest/background site, algae were present on lichens in the trees (Figure 4), even at this site which is much more distant from immediate local sources. This is a sign of N-enrichment from atmospheric sources.

Figure 4: Lichen (*Evernia* sp) covered with algae close to site S8 (background site, away from immediate local emission sources). Photograph by Netty van Dijk

It was notable that across the bog, healthy and damaged vegetation of the same species were often found very close together, with some examples shown in Figures 5 and 6. This may be due to several reasons: local sheltering, differences in sensitivity between individual plants (possibly genetic) and different abilities of individual plants to adapt to higher N levels.

Grazing by cattle or sheep can cause these very local differences in damage of vegetation. However, as there is no grazing at Ballynahone Bog this can't be part of the explanation.

Figure 5: Very unhealthy (right), covered with algae and healthy (left) *Cladonia portentosa* very close to each other near S7. Photograph by Netty van Dijk

Figure 6: Healthy (right) and damaged (left) *Sphagnum* near site S6. Photographs by Netty van Dijk

On 5th March 2019, two weeks before the vegetation sampling was undertaken on18 and 19 March 2019, a pink colouring of *Cladonia* was observed on the bog, especially near site S3. On the sampling dates, this colouring had disappeared and the *Cladonia* was bleached and brittle, which can be seen as the next stage of N-damage in *Cladonia*. Around site S5 there is more grass in the vegetation than at other sampling sites on the bog, which is a sign of N-enrichment. Near farm 2, nettles, brambles and grasses were prevalent on the bog, a clear indication of N-enrichment. In Appendix I,

a set of photographs provide more examples of observations at the sample locations near the NH_3-air monitoring sites, as well as some examples of typical visual N-damage to the vegetation (*Polytrichum*, *S. capillifolium* and *Cladonia*).

3.2. Biomonitoring

At some of the vegetation sampling sites it was difficult to find sufficient plant material from the species under investigation.
Sphagnum (x2) and *Hypnum* were collected at all/most of the sites; *Cladonia* was only found at 9 of the 15 locations and was often in poor condition (Figure 7).
At site S1, only two of the four target species were found. *Pleurozium* was collected as a substitute at the time, although it was unknown whether the same substitute would be required for other sites (the alternative option would have been not to collect any substitutes).
An overview of collected samples can be found in Table 1 of Appendix III.

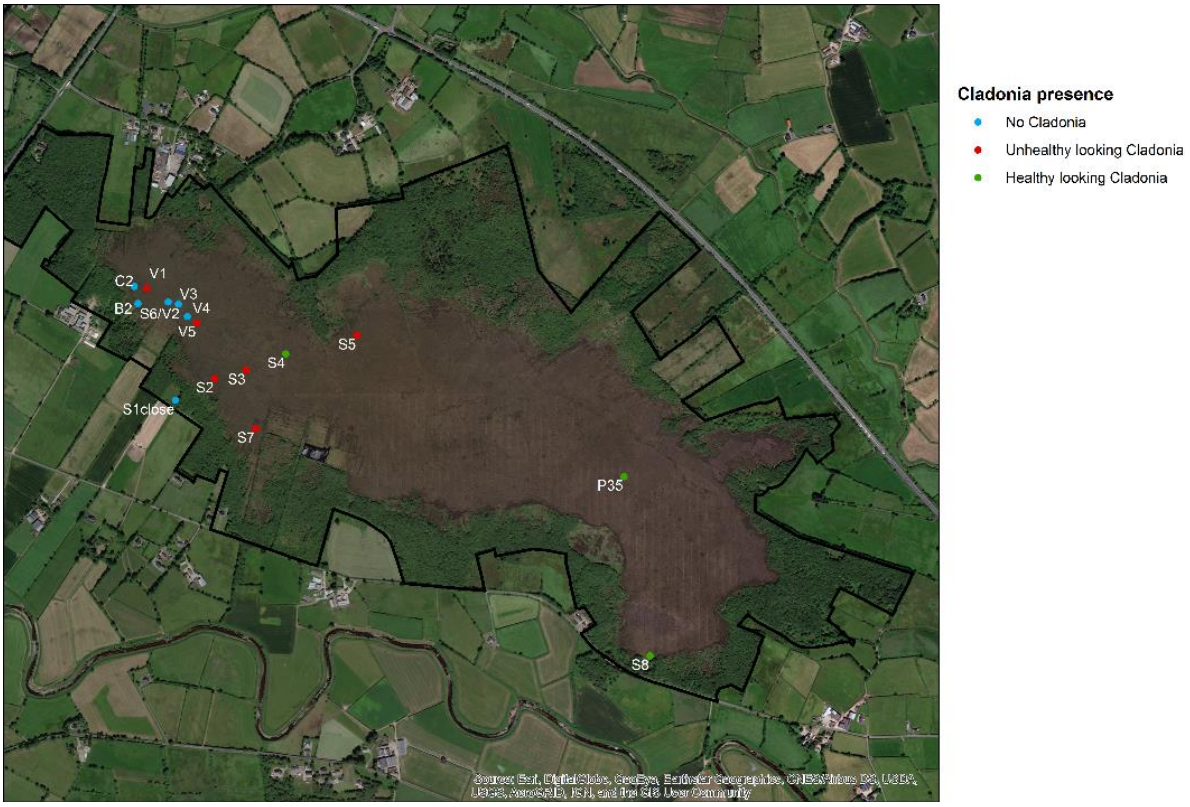

Figure 7: Overview of presence and healthiness of *Cladonia* at the vegetation sampling sites. S5, S8 and P35 are the 'reference sites', which are furthest away from the farms.

%N DW	*S. capillifolium*	*S.subnitens*	*Hypnum*	*Cladonia*	*Pleurozium*
site					
C2	1.07	-	1.73	-	-
B2	1.32	1.12	1.52	-	-
V1	1.42	1.23	1.42	0.74	-
V2 (S6)					
V3	1.3	0.95	1.3	-	-
V4	1.14	1.01	1.15	-	-
V5	1.21	0.9	1.12	0.68	-
S1	-	-	-	-	-
near S1	1.06	-	1.31	-	1.05
S2	1.34	0.99	1.33	1.2	-
S3	0.88	0.94	1.15	0.75	-
S4	1.16	1.01	1.9	0.9	-
S5	1.06	0.93	2.1	0.98	-
S6 (V2)	1.01	1.1	1.05	-	-
S7	1.2	0.88	1.31	0.88	-
S8	0.88	0.85	0.84	0.64	-
P35	1.02	0.77	1.36	0.6	-

Table 1: %N (DW) in vegetation samples. '-' indicates that no or not enough plant material was found. S5, S8 and P35 (green shaded areas) are the 'reference sites', which are furthest away from the farms.

NH4-N	*S. capillifolium*	*S.subnitens*	*Hypnum*	*Cladonia*	*Pleurozium*
(µg g-1) FW					
C2	**5.03**	50.80	139.44	-	-
B2	29.73	**14.72**	337.30	-	-
V1	7.59	21.96	127.84	234.99	-
V2 (S6)					
V3	**29.76**	**7.92**	62.71	-	-
V4	15.33	**11.45**	88.42	-	-
V5	6.31	**11.98**	48.75	18.00	-
S1	8.53	-	-	-	
near S1	**15.43**	-	**48.32**	-	24.41
S2	12.63	**12.04**	47.37	**142.53**	-
S3	7.58	**13.07**	**128.40**	**230.59**	-
S4	**22.07**	34.10	464.39	308.22	-
S5	**9.06**	**8.61**	**369.91**	80.10	-
S6 (V2)	15.08	**8.79**	**14.20**	-	-
S7	**27.11**	*	**34.01**	**79.85**	-
S8	**20.56**	17.04	**49.22**	**98.10**	-
P35	**12.71**	**11.41**	131.47	**14.59**	-

Table 2: NH_4-N FW in vegetation samples, '-' indicates no or not enough plant material present. * Indicates sample lost during processing. Numbers in bold are an average of 2 subsamples. Other numbers are from a single sample. S5, S8 and P35 (green shaded areas) are the 'reference sites,' which are furthest away from the farms.

It is notable that at two (S5 and P35) of the three sampling sites furthest away from the farms (S5, S8 and P35) the %N and foliar NH_4-N found in *Hypnum* are quite high

(Tables 1 and 2, Figure 8). Also, levels are relatively high in the *Sphagnum* samples at site S8.
At S5, there was quite a lot of grass growing, which is also an indication of N-enrichment. This might suggest that there are additional N-sources impacting the vegetation.
It is not solely NH_3 air concentrations which contribute to %N in the vegetation; N-concentrations (NH_4^+ and NO_3^-) in precipitation (rain) also contribute in the form of wet deposition. Although we expect the wet deposition to be relatively constant across the site, it would be a particularly useful addition to have a wet deposition collector at Ballynahone Bog.
The different vegetation species react in distinct ways to NH_3 and the high concentrations in *Hypnum* are an indication that *Hypnum* can 'hold' higher levels of NH_4-N before it is visually damaged.

For many samples, there was not sufficient material to split the sample and analyse two sub-samples for NH_4-N; however, where duplicate analysis was possible, the number in Table 2 is an average (bold numbers). Full, detailed results are shown in Table 2 of Appendix III. Even though subsamples were taken from the same main sample, sometimes there was a rather large difference between duplicates.
Table 2 shows that there is a big variation between the foliar NH_4-N concentrations of the samples.

In an attempt to make this foliar NH_4-N ($\mu g\ g^{-1}$) FW measurement more robust and easier to compare with %N, we have estimated the NH_4-N ($\mu g\ g^{-1}$) DW (dry weight) by determining the %dry mass of the left-over subsamples and applied this % to the amount of frozen samples used in the NH_4-N analyses. The % dry mass was very similar for both the *Sphagnum* mosses with an average of 14.2 % (minimum 9.3% and maximum 23.4%) for *S. capillifolium* and an average of 13.2% (minimum 8.0% and maximum 27.0%) for *S. subnitens*. For *Hypnum,* the percentage dry mass was slightly higher with an average of 21.9% (minimum 15.6% and maximum 36.7%) and for *Cladonia* it was higher again with an average of 42.7% (minimum 20.4% and maximum 59.6 %; see Table 3 in appendix III for the details). We applied these %dry mass to the frozen weight of the (sub-)samples which were used to analyse for NH4-N. In this way we obtained an estimate of the NH_4-N ($\mu g\ g^{-1}$) DW (dry weight), see Table 4 in appendix III for the full list.

Table 3 shows the ratio between NH_4-N ($\mu g\ g^{-1}$) FW fresh weight and the estimated NH_4-N ($\mu g\ g^{-1}$) DW dry weight. As with the % dry mass, the DW/FM ratio is very similar for both *Sphagnum* mosses with an average of 0.16 (minimum 0.10 and maximum 0.25) for *S. capillifolium* and an average of 0.15 (minimum 0.09 and maximum 0.29) for *S. subnitens*. For *Hypnum* the DW/FW ratio is 0.24 (minimum 0.17 and maximum 0.39) and for *Cladonia* 0.45 (minimum 0.22 and maximum 0.62).

FW/DW ratio	*S. capillifoliu*	*S. subnitens*	*Hypnum*	*Cladonia*	*Pleurozium*
site					
C2	0.17	0.16	0.27	*	*
B2	0.25	0.12	0.17	*	*
V1	0.17	0.12	0.18	0.22	*
V2 (S6)					
V3	0.12	0.16	0.39	*	*
V4	0.15	0.29	0.17	*	*
V5	0.18	0.16	0.37	0.57	*
S1	*	*	*	*	*
near S1	0.16	*	0.21	*	0.31
S2	0.15	0.15	0.17	0.45	*
S3	0.16	0.10	0.24	0.31	*
S4	0.12	0.12	0.21	0.43	*
S5	0.11	0.13	0.21	0.58	*
S6 (V2)	0.21	0.19	0.18	*	*
S7	0.11	*	0.32	0.35	*
S8	0.10	0.09	0.20	0.62	*
P35	0.19	0.12	0.25	0.51	*

Table 3: Ratio between NH_4-N (µg g^{-1}) FW fresh weight and the estimated NH_4-N (µg g^{-1}) DW dry weight for the Ballynahone vegetation samples. * stands for no sample available

In theory, if the NH_4-N (µg g^{-1}) DW dry weight and the %N (also based on dry weight) is known, it is possible to estimate how much NH_4-N (µg g^{-1}) DW contributes to %N. However, for this study this would be too speculative, as we have so many estimates and only a very limited number of samples. For future studies, it would be good to determine the %dry mass of each sample used for analysing foliar NH_4-N.
Estimating the NH_4-N (µg g^{-1}) DW did not decrease the variation in foliar NH_4-N very much. To increase the robustness of this foliar NH_4-N method, an increase in number of samples would be advisable.

NH_4-N (μg^{-1} FW)

- < 10
- 20 - 50
- 50 - 100
- 100 - 200
- > 200
- NA

% N DW

- < 1.00
- 1.00 - 1.25
- 1.25 - 1.5
- 1.5 - 1.75
- > 1.75
- NA

Figure 8: %N (right) and foliar NH_4-N concentration FW (left) in, from top to bottom, *S. capillifolium*, *S. subnitens, Hypnum*, and *Cladonia* at the different sample locations. NA refers to no data being available for a species.

The vegetation samples taken at the NH_3 concentration measurement sites often have their highest concentrations at S3, S4 and S5, even though these are not the highest NH_3 air concentrations. This indicates that it is not only farm 1 that contributes to the vegetation damage. During the site visit it was noticed that at S5, more grass was growing in comparison with other sites, which is an indication of N-enrichment. The highest average NH_3 air concentration during 2018 was measured at site S6 (Figure 9). It would be interesting to have an additional NH_3 air monitoring point along a transect away from farm 2. Detailed information about the NH_3 concentrations at the eight monitoring sites can be found in Appendix IV.

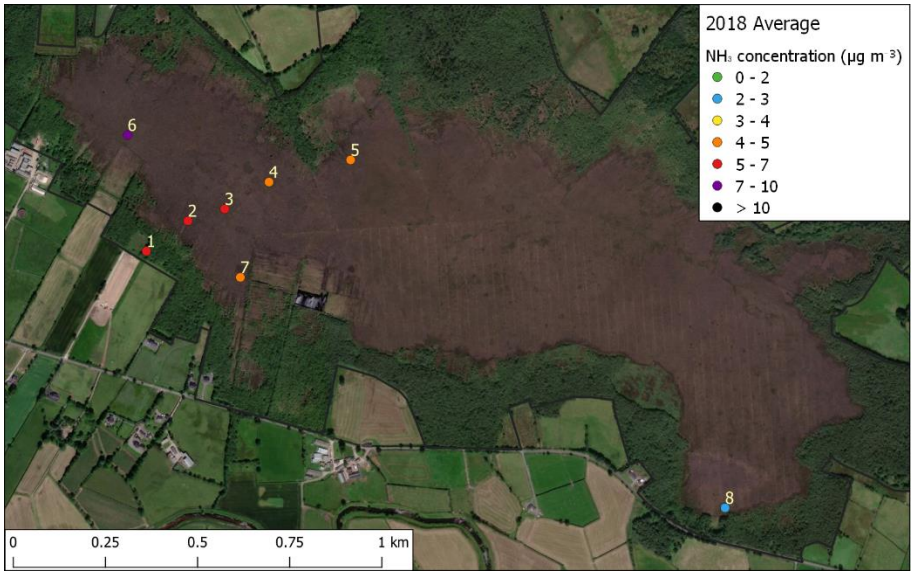

Figure 9: Average NH_3 air concentration (µg m^{-3}) measured in 2018. From Tang et al. 2019

A point to consider is that around sites S1 and S2, close to farm 1, the vegetation is different from the rest of the bog. There are more shrubs, trees and taller plants present and more hummocks and hollows. This means that the moss species are more sheltered from NH_3. It should also be noted that the NH_3 measurements are carried out at the standard height of 1.5m whereas the mosses are sampled at ground level. From the Whim Bog manipulation site, we know that the measured NH_3 concentrations at vegetation level only slightly differ from the measured concentrations at 1.5m height. Another point to note is that the emissions from farm 1 during operational conditions are from a high stack (16 m above ground level), whereas ground level emissions are expected during cleaning operations between cycles when the farm is destocked.

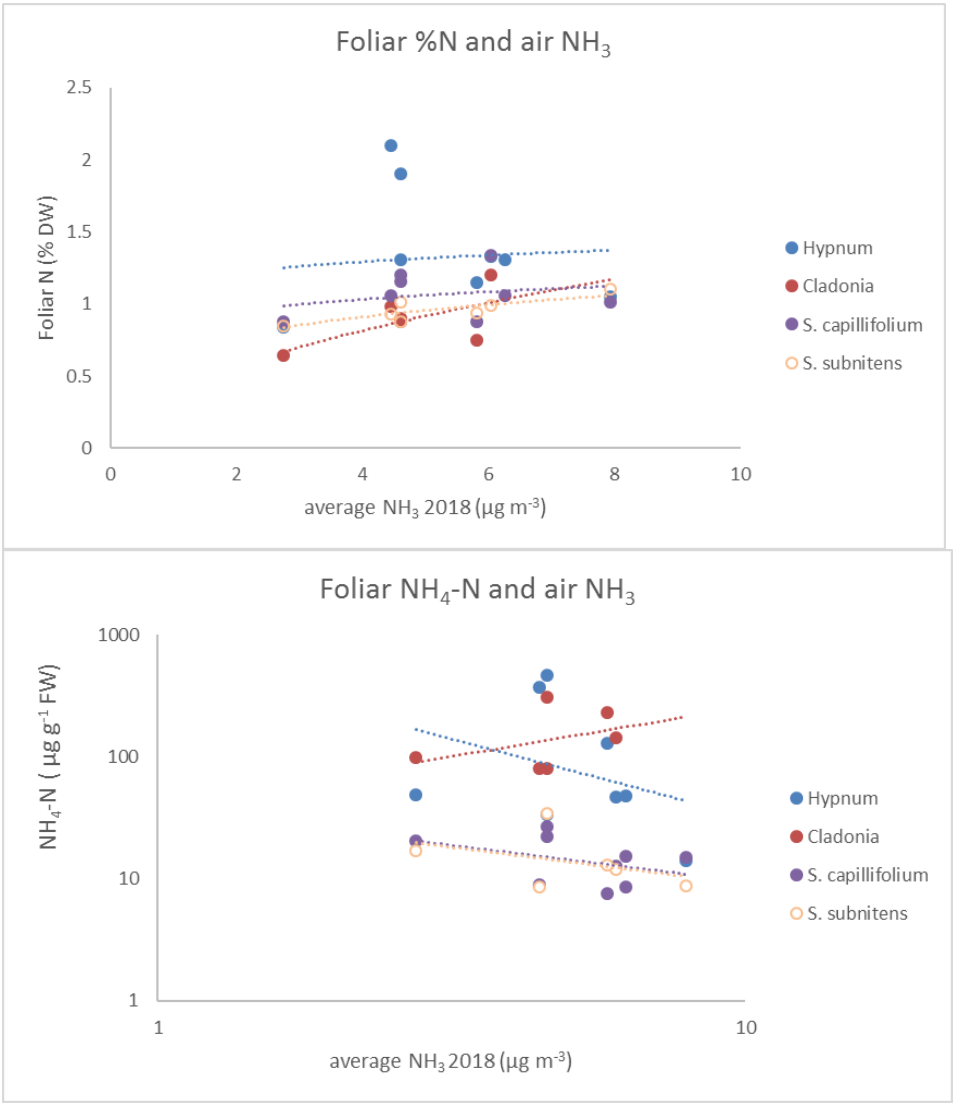

Figure 10: %N (top graph) and NH_4-N (µg g^{-1} FW) (bottom graph) and NH_3 air (µg m^{-3}) concentration (average of 2018).

For *Sphagnum subnitens* there was a good correlation (r^2 =0.69) between %N in the vegetation and the average atmospheric NH_3 concentration over 2018, whereas for *Cladonia* there was only a weak correlation. This is in line with previous studies (Leith et al 2005, JNCC report 386), which also showed that different plant species react differently to an increase in NH_3 concentrations.

There was only a very weak correlation between NH_4-N and the atmospheric NH_3 concentration during 2018. This might be due to the very high NH_3-air concentrations during the period Oct-Dec 2018 and March 2019 when the samples were collected, and related NH_4-N saturation in the plants.

NH_4-N is more sensitive to changes in the NH_3 air concentrations than %N. The NH_4-N found in the Ballynahone Bog samples was much higher than we have found in previous research (Leith et al 2005, JNCC report 386) and at Moninea Bog SAC. (Van Dijk et al. in preparation).

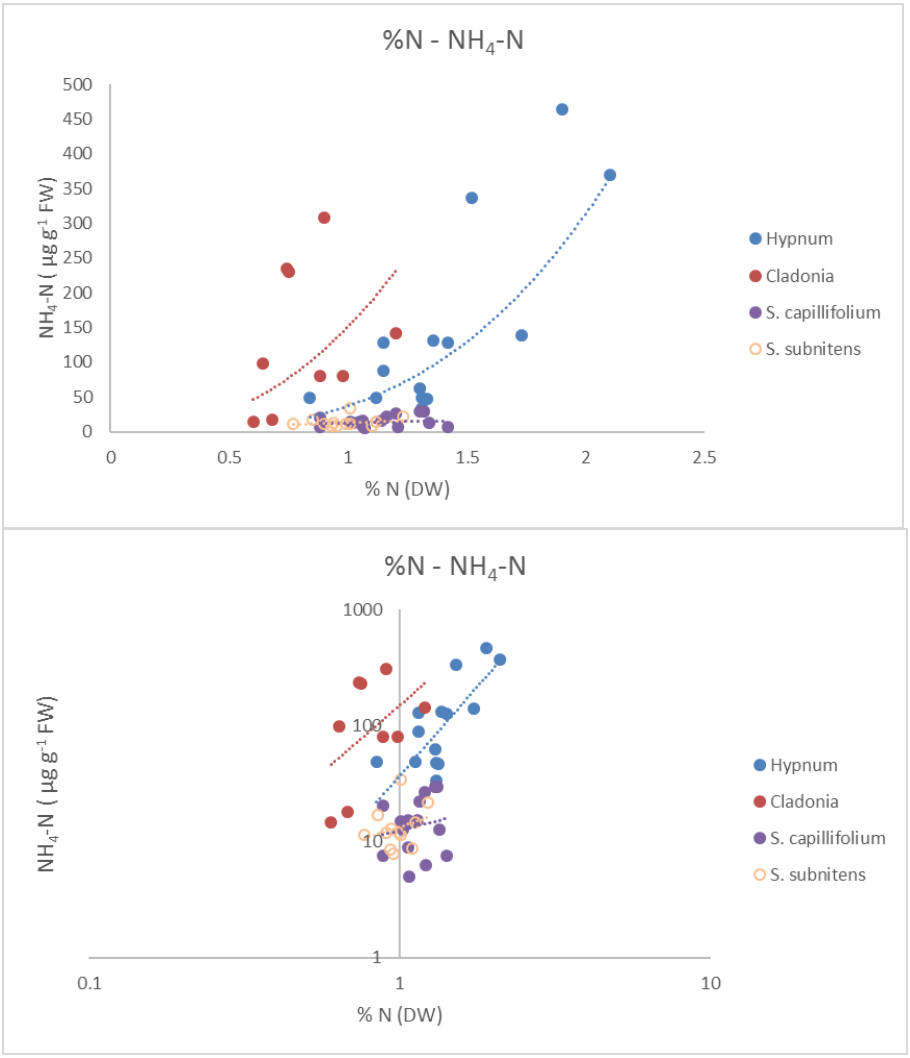

Figure 11: relation between %N and NH4-N; top graph on normal scale which is easier to explain; bottom graph on log-scale.

Figure 11 shows that the different vegetation species react in distinct ways, however both the *Sphagnum* species (*S. capillifolium* and *S. subnitens*) behave in a similar way. Only for *Hypnum* is there a good correlation between %N and NH_4-N. This is in line with previous research (Leith et al 2005, JNCC report 386); a strong correlation was only found when there was a distinct NH_3 point source. This is an additional indication that there are different NH_3 sources around Ballynahone Bog which all affect the vegetation on the bog. There is not much data available about these species-specific concentrations and it is also unknown why the different species react in different ways.

3.3. Comparison of Ballynahone Bog with other sites in Northern Ireland

A comparison was carried out of the samples analysed for Ballynahone Bog with other sites in Northern Ireland, Lough Navar and Moninea Bog. These sites are quite different from Ballynahone Bog in terms of ammonia concentrations. Lough Navar is a clean site in the west of Northern Ireland with low NH_3-air concentrations of 0.7 -1.0 µg

m^{-3} in 2007 and consequently has low concentrations in the vegetation samples. It is represented here as a clean reference site.

Moninea Bog, in the southwest of Northern Ireland, was affected by a farm that caused high local NH_3 concentrations. The farm was the only large NH_3 source close to the bog, with high NH_3 concentrations (>25 µg m^{-3}) being recorded, which dropped dramatically to 1.7 -2.0 µg m^{-3} at 840 m away from the farm in 2007 (Sutton et al 2011, Natura 2000 book, Van Dijk et al *in preparation*) and then dropped even further to a low background. Therefore, a clear NH_3 gradient along the bog from west to east could be identified. The farm ceased operations in 2010, and in 2017 the measurements were repeated at the same locations, ten years after the initial study.

By contrast, there are several NH_3 point sources at Ballynahone Bog, with relatively high background NH_3 air concentrations even at the background site, furthest away from these sources (S8, >2 µg m^{-3}). There are no clear gradients in NH_3-air concentrations when the levels are high, and specifically during the months preceding the sampling. Therefore, the lack of a clear gradient in %N and NH_4-N in the vegetation samples is expected.

The results show how the sites are impacted differently, depending on the particular situation at each site and the make-up of emission sources (e.g., is there one or more point source(s) or are there several diffuse sources). This makes it difficult to directly compare the results from one site with another. One of the differences between the sites is that there are different vegetation species present, so it was not possible to compare on a species level. All species from each site were grouped together here for comparison (Figure 12). Only data from sample locations where NH_3 concentrations were also available were used in the comparison.

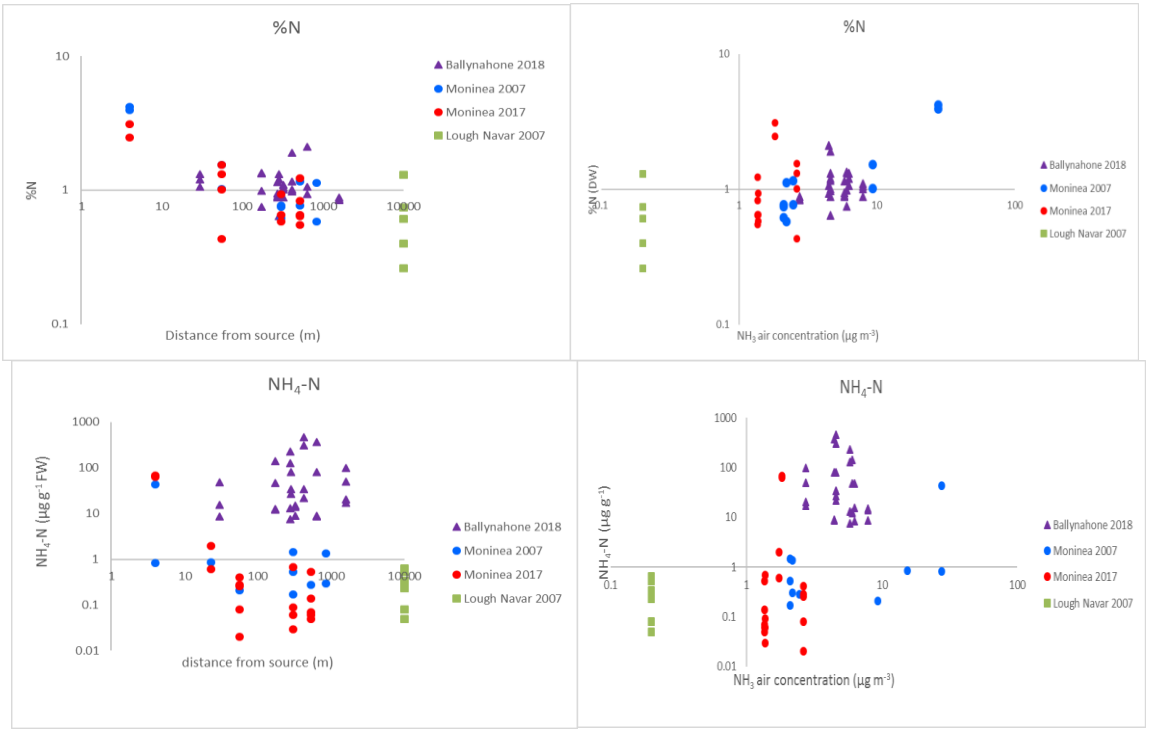

Figure 12: %N (top) and NH_4-N (bottom) in relation to distance from the source (left) and NH_3 air concentration (right) for Ballynahone (2019), Moninea (2007 and 2017) and Lough Navar (2007). Lough Navar is presented on an indicative 10km away from a source.

As expected, the results from the clean reference site, Lough Navar, are much lower than the results from the other two sites. This is the green cluster (squares) in Figure 12. There is no direct NH_3 source near the site, which results in low NH_3 air concentrations and therefore in low %N and low foliar NH4-N in the vegetation. The %N found in the vegetation samples at Ballynahone Bog are in the same range as the %N found at Moninea Bog. However, the foliar NH_4-N found in the vegetation at Ballynahone Bog (purple cluster, triangles in Figure 12) is much higher than the levels found at Moninea Bog. This might be due to the high NH_3 concentrations in Oct-Dec 2018 and March 2019. As mentioned earlier, the foliar NH_4-N concentrations react much quicker to changes in air concentrations than %N. The highest concentrations at Moninea Bog were found in a woodland area close to the former poultry farm, with different species present (more sensitive species were absent, presumed extinct). The NH_3 air concentrations at Moninea Bog have decreased dramatically between 2007 and 2017. The decrease in foliar NH_4-N and %N over the same period was small, which is an indication that there is still N present in the system.

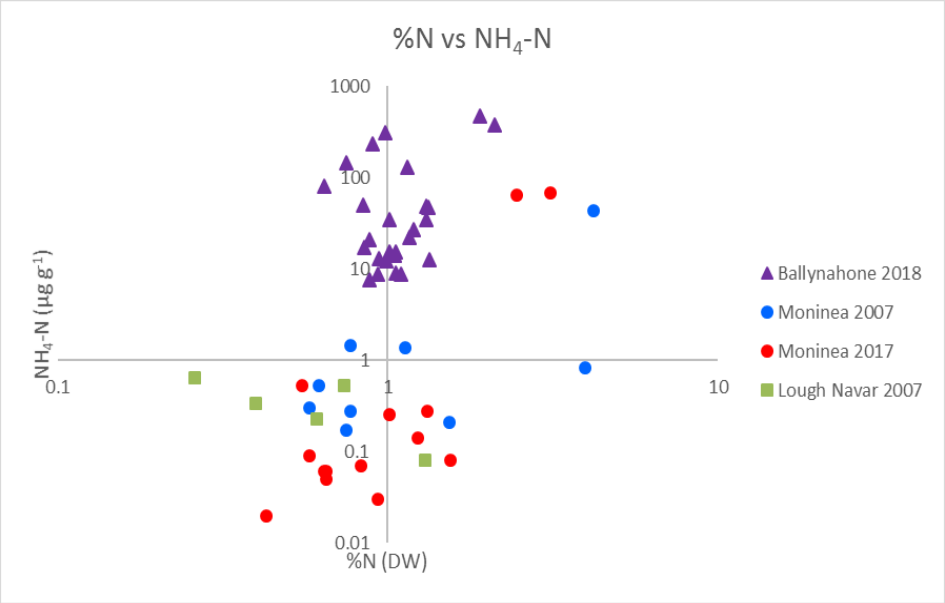

Figure 13: Relationship between %N and NH_4-N for Ballynahone Bog (2019), Moninea (2007 and 2017) and Lough Navar (2007).

Figure 13 illustrates again that foliar NH_4-N at Ballynahone Bog (purple cluster) is high relative to the other sites. It also illustrates that the foliar NH_4-N concentrations at Moninea Bog have dropped more relative to the %N between 2007 and 2017.

3.4. NH_3 air concentration

During the collection of the vegetation samples in March 2019, there was a strong smell at the western end of the bog.

This was also evidenced with the monitored concentrations during Oct-Dec 2018 and at some monitoring sites in March 2019, with relatively high levels of ammonia recorded. Appendix IV shows detailed NH_3 concentration information for this period. This high concentration episode is thought to have had a substantial effect on the results of the vegetation analysis. Foliar NH_4-N will change more quickly than %N, but there is currently little evidence available on how quickly these changes occur, and how this may differ between plant species. It might also be possible that in some plants the foliar NH_4-N is near to saturation. There are some hypotheses that foliar NH_4-N concentrations in plants have a maximum level which may be different between species. In other words, NH_4-N concentrations in the plant cannot exceed this maximum level, even if atmospheric NH_3 continues to increase. However, to our knowledge, this has not been proven.

%N reacts more slowly to changes in NH_3 air concentrations but again, there is, to our knowledge, no evidence on how quickly/slowly this reaction occurs. It is possible that this is a slower process, with longer term concentrations playing a role, rather than the previous few months' concentrations. This might suggest that %N and NH_4-N are complementary biomonitoring tools that provide insights on different time scales.

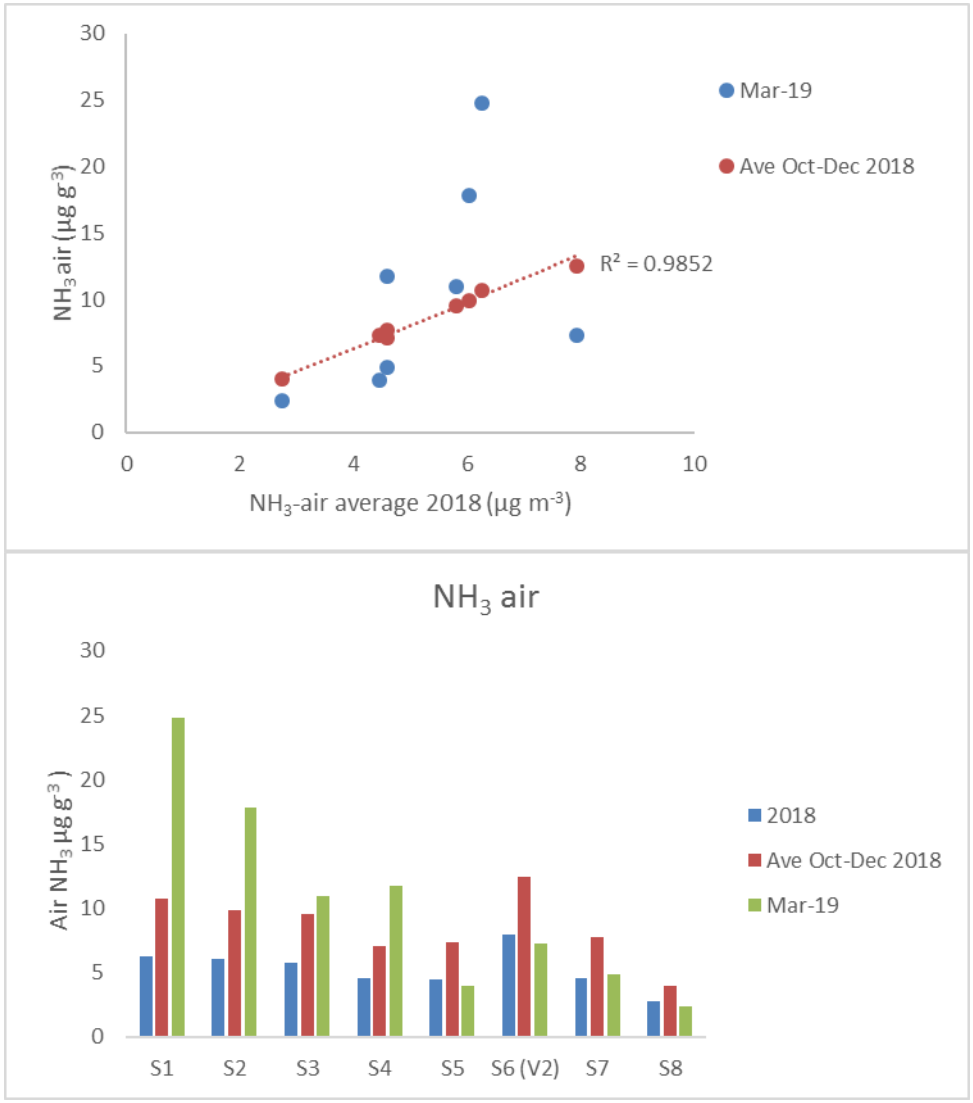

Figure 14: Average NH_3 air concentrations in Oct-Dec 2018 and March 2019 against average NH_3 air concentrations in 2018 (top) and the NH_3 concentrations at each of the monitoring sites (bottom).

The average NH_3 air concentration for Oct-Dec 2018 has a high spatial correlation with the average of the whole of 2018; only in Oct-Dec are the concentrations much higher (Figure 14). At site S6, the Oct-Dec 2018 concentrations are the highest out of all the sites, indicating that farm 2 plays a role.

For March 2019, there was another peak in NH_3 air concentrations, however the spatial pattern was different. Sites S1, S2 and S4 had particularly higher concentrations than the average of 2018.

Wind direction is an important factor which provides better insight into which sources contribute at what time to the measured NH_3 air concentration, as well as to establish if there are other sources that might be missed by the air measurements. Wind measurements are being considered as part of another task in the wider Ballynahone Bog project (task 1).

4. Conclusions

The vegetation on Ballynahone Bog is in general in quite poor condition. Even at the sample sites furthest away from the known NH_3 sources, there are visual signs of N damage, which is supported by the high N content (%N) and high foliar NH_4-N (FW) concentrations found in the vegetation.
At a micro-scale, the health of the vegetation is very variable across the site. Plants with clear visual damage and apparently healthy plants are found close to each other. This variability between samples taken was also found in the bioindicator %N and foliar NH_4-N (FW), even when the samples were all healthy looking. This makes the interpretation of the data much more complicated. To get clearer information about the status of the plants, a larger vegetation sample set needs to be collected and analysed.
Although the %N at Ballynahone bog and Moninea Bog are in the same range, the foliar NH_4-N concentrations at Ballynahone Bog are much higher than at Moninea Bog. Both the %N and the NH_4-N indicators at Ballynahone Bog are much higher than in vegetation from a clean site, such as Lough Navar.
There are remarkably high foliar NH_4-N concentrations found in the vegetation at Ballynahone Bog in comparison with other sites. It is likely that this has been caused by very high NH_3 air concentrations during the months immediately preceding the sample collection. Foliar NH_4-N concentrations in the vegetation react faster to temporarily high concentrations than %N.
Although the results of this study are complex, they clearly show that the high NH_3 concentrations, both across the site and during the high concentration episodes, caused by the local sources, have N-related impacts on the vegetation at Ballynahone Bog.

5. Recommendations

It would be interesting to investigate further how the NH_3 air concentrations change along a transect away from farm 2 (assuming prevailing wind) and how they interact with the NH_3 concentrations along the transect away from farm 1. To separate the NH_3 emission contributions from both farms, more NH_3 measurements are necessary and detailed data for wind direction and wind speed are essential. It would also be interesting to measure %N and NH_4-N in the vegetation in relation to distance from farm 2 and combine this with the information about the effects from farm 1.

Air concentration data measured with the ALPHA samplers only become available 2-3 months after the measurement period, due to shipping and laboratory analysis being required. Therefore, it is only possible to systematically quantify the NH_3 concentrations later (apart from that which could be smelled whilst sampling). Vegetation samples need to be collected at the end of the winter/early spring before plant growth begins and the N in the vegetation becomes diluted. Therefore, unfortunately, sampling at a few months' intervals and later deciding which samples to analyse (once the air concentrations are known) is not possible/practical.
Repeat sampling of vegetation a few months apart will also reduce the amount of suitable vegetation samples at the site, leaving insufficient material for frequent re-sampling. Only the heads (*Sphagnum*) or the head tips (bryophytes) can be used for

analysis. A sufficient amount of vegetation is required for each sample, which needs to be sorted and cleaned before use. Unclean and unsorted samples deteriorate after a while, even when stored in cold-room conditions. Collecting and, to a greater extent, sorting/cleaning is very time consuming, meaning that it would not be an efficient use of resources to prepare all repeat samples before deciding which ones to use.

Although foliar NH_4-N, and to a lesser extent %N, are affected by local NH_3 air concentrations, other forms of N deposition such as wet N deposition in precipitation and oxidised N deposition (from NO_x emissions) can also play a role. To get a better understanding of all types of N deposition at the site, it would be especially useful to install a wet deposition (rain) collector and a NO_x concentration sampler at the site and analyse the content for the other main N forms.

A further recommendation could be to carry out an experiment in an open top/ vegetation chamber. This involves fumigating with NH_3 over a chosen vegetation type (moss, *Sphagnum*) and would require growing/transplanting the plants before the start of the experiment. This would also require a dose system where amounts of NH_3 can be clearly quantified. Harvesting of plants would be required before the start of the growing season/or continuously, followed by analysis for NH_4-N and %N. This would be a substantial project on its own but could provide some indication of how quickly nitrogen concentrations in the plant (NH_4-N and %N) react to changing NH_3 air concentrations.

6. References

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7. Appendices

Appendix I: Vegetation at the air-monitoring sites at Ballynahone Bog and examples of N-damage at *Polytrichum*, *Sphagnum* and *Cladonia*.

Photographs by Netty van Dijk, unless noted otherwise

Site 1: At the edge of the bog, near farm 1.

Between Site 1 and 2 Hummocks and hollows:

Vegetation on the hummocks covered with algae; in the hollows healthy looking *Sphagnum*.

Site 2: ALPHA sampling post

Site 3
NH3 air-measurements with ALPHA and DELTA samplers, power for DELTA from solar panel and small windmill.

Pink coloured *Cladonia*; N-damage; in this stage damage is still reversible (photographs by Áine O'Reilly). At the right *Cladonia* bleached and start to disintegrate.

Site 4
No photograph

Site 5:
More grassy area, ALPHA sampling post; collecting vegetation samples

Site 6:

Left: ALPHA sampling post; healthy (middle) and damaged (right) *Sphagnum* found very close to each other



Site 6 towards farm 2 at the edge of the bog

N-loving vegetation towards farm 2**.**


Healthy, bright green *Polytrichum*, the left branch in the bottom photograph; the right branch (in the same photograph) is a much darker green *Polytrichum*, which is a sign of N-enrichment.

Site 7

Left: healthy *Cladonia*; middle: pink coloured, sign of N-damage, but still possible to recover; right: damaged, covered with algae

Site 8

Close to site 8 (furthest away from both farms):

.

For comparison, photographs of lichens on trees at the clean site Lough Navar.

Left photograph by Ian Leith; right photograph by Áine O'Reilly.

N damage *Polytrichum*

Top: healthy *Polytrichum.* Bottom: damage by Nitrogen, left browning and on the right very dark, almost black *Polytrichum* (bottom photographs by Ian Leith)

Sphagnum visible damage symptoms by ammonia

'Clean '

Initial damage

Capitulum loose

Disintegration and surface slime

Loss of capitulum structure & slime

Final disintegration

Cladonia portentosa visible damage symptoms

Ammonia

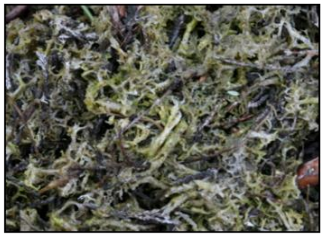
'clean'

'Pinking' due to NH_3 & strong sunlight with 5 weeks. Reversible.

Bleaching of apices & increase in algae in the necromass

Bleaching and early stage of disintegration of the apices

Loss of apical structure



Total disintegration and increase in algae

Photographs Sphagnum and Cladonia damage by Ian Leith

APPENDIX II

Protocols for collecting and processing vegetation samples Ballynahone

ALWAYS WEAR GLOVES WHEN HANDLING THE VEGETATION SAMPLES
This is to avoid contamination of the sample with N (and other acids/fats) from your skin.

Collecting vegetation samples in the field.

- Collect the same species at the different sites, as much as possible.
- Collect from healthy looking plants, if possible
- Collect the samples in the field as 'clean' as possible: no other species, grass, leaves, soil and parts of the plant you don't need. This will save time later on when you are cleaning/sorting the sample.
- Put in a plastic bag, label them with species name, site name/number and collecting date.
- Store in a cool place (fridge, coldroom) at about 4°C until you can sort and clean them.

Sorting and cleaning vegetation samples:

- Only use the young ends (top 1.0 – 1.5 cm) of the shoots or for *Sphagnum* only the heads/capitula of the chosen species.
- Take out all other species/leaves/wood/soil.
- Rinse the sample with a little de-ionised water (a sieve is useful for this) to remove smaller bits of soil/leaves.
- Blot-dry the sample (don't press too hard), you may need to use a few sheets of paper towel/blue roll for this.

At this stage split the sample for %N and soluble ammonium (NH_4-N). Make sure that the sample is mixed very well before doing that.

Preparation of vegetation sample for %N analysis

- Put the sample in a small paperbag, label the bag and dry it in the oven at 60 °C (not warmer) for 24 hours. If your sample is very big you may need to dry them longer. You can air-dry (just leave the samples at room temperature) the sample first if you are not able to use the oven immediately)
- Let the samples cool down and store the dried samples at room temperature before you go to the next step of grinding the sample.
- Grind the sample using a ball mill until a fine powder.
- Put the powder in a small glass vial and close the lid. (We are using 7ml glass vials). Clearly Label the vials. Ideally fill the vial for at least half with your sample.
- Send them to the lab for %N analysis. If you cannot send them immediately store them at room temperature.

%N analysis

Samples were analysed for %N content at CEH Lancaster using a Vario-EL element analyser.

Preparation of vegetation sample for soluble ammonium NH_4-N

It is important that the samples are as blot-dry as possible but don't press the samples. You need a minimum of 2 grams of sample, so try to store a bit more.

- Put them in a plastic bag, label and store in a freezer at -20°C until you extract them for NH_4-N analysis.

Extracting vegetation samples and analysis for NH_4-N

- Weigh 2g FW of frozen, cleaned vegetation material and put it in a sample pot. Write down the exact amount.
- Add 20ml de-ionised water.
- Shake for 5s and leave to extract for 4h at lab temperature (20°C).
- Shake again for 5 s.
- Filter solution through 0.45 µm pore syringe filter.
- Store solution in freezer at -20°C until analysis.
- Analyse the sample following the standard protocol (same as for ALPHA samples) on the SEAL AA3 analyser. The SEAL AA3 is an instrument utilising a colorimetric reaction to detect the concentration of ammonium in water (modified Berthelot). It is important to note the reaction chemistry is specifically corrected for the impact of the low pH of the sample.

Calculating the NH_4-N concentration in the vegetation samples FW (fresh weight)

NH_4-N (µg g^{-1}) FW

$$N_{f,NH4\text{-}N} = [N_{NH4\text{-}N} - N_{blank}] * (M_{extractant} + M_{f,sample}) / M_{f,sample} \quad (1)$$

Where:

$N_{f,NH4\text{-}N}$ is the soluble NH_4-N concentration (µg g^{-1} fresh weight (FW)) in the vegetation sample

$N_{NH4\text{-}N}$ is the NH_4-N concentration in the extraction solution (µg g^{-1})

N_{blank} is the NH_4-N concentration in the blanks (µg g^{-1})

$M_{extractant}$ is the mass of extractant (de-ionised water) used (20 g as standard)

$M_{f,sample}$ is the fresh mass (FM) of the vegetation sample (g)

Estimating the NH_4-N concentration in the vegetation samples DW (dry weight)

NH_4-N (µg g^{-1}) DW

The left-over frozen samples were weighed, dried in the oven at 60° C for 48 hours and weighed again. This % dry mass is then applied to the fresh mass of the used sample to estimate the NH_4-N concentration dry weight using the same calculations as for fresh weight (see above (1)).

APPENDIX III: Ballynahone Bog sample information

Site number	*S. capillifolium*	*S. subnitens*	*Hypnum*	*Cladonia*	*Pleurozium*
C2	X	X	X	_	
B2	X	X	X	_	
V1	X	X	X	X	
S6 =V2	X	X	X	_	
V3	X	X	X	_	
V4	X	X	X	_	
V5	X	X	X	X	
S1close	X	_	X	_	X
S2	X	X	X	X	
S3	X	X	X	X	
S4	X	X	X	X	
S5	X	X	X	X	
S7	X	X	X	X	
S8	X	X	X	X	
P35	X	X	X	X	

Table 1: Vegetation samples collected at Ballynahone Bog on 19 and 20 March 2019. '-' means no or not enough vegetation available for sampling. A light blue background means that only a small sample has been collected as there was not enough vegetation available. In the *Cladonia* column a pink background means the vegetation was in a poor condition and a light green background means the *Cladonia* looked healthy. At S1 no mosses were found, only brambles and nettles; the vegetation has been collected as close as near S1 as possible. *Pleurozium* has only collected near S1 as a substitute.

NH4-N fresh weight	*S. capilllifolium*			*S. subnitens*			*Hypnum*			*Cladonia*			*Pleuroziur*
site\ NH4-N(µg g-1 FW)	A	B	mean	A	B	mean	A	B	mean	A	B	mean	A
C2	1.65	8.41	**5.03**	50.80	-	**50.80**	139.44	-	**139.44**	-	-	-	-
B2	29.73	-	**29.73**	13.43	16.01	**14.72**	337.30	-	**337.30**	-	-	-	-
V1	7.59	-	**7.59**	21.96	-	**21.96**	127.84	-	**127.84**	234.99	-	**234.99**	-
V2 (S6)													
V3	32.74	26.78	**29.76**	7.82	8.02	**7.92**	62.71	-	**62.71**	-	-	-	-
V4	13.45	17.21	**15.33**	12.39	10.50	**11.45**	88.42	-	**88.42**	-	-	-	-
V5	6.31	-	**6.31**	6.85	17.11	**11.98**	48.75	-	**48.75**	13.42	22.58	**18.00**	-
S1	11.84	5.21	**8.53**	-	-	-	-	-	-	-	-	-	-
near S1	11.76	19.10	**15.43**	-	-	-	44.44	52.20	**48.32**	-	-	-	**24.41**
S2	12.63	-	**12.63**	12.41	11.67	**12.04**	47.37	-	**47.37**	36.29	248.77	**142.53**	-
S3	5.10	10.05	**7.58**	13.25	12.89	**13.07**	92.71	164.08	**128.40**	297.85	163.32	**230.59**	-
S4	19.62	24.52	**22.07**	34.10	-	**34.10**	464.39	-	**464.39**	308.22	-	**308.22**	-
S5	6.02	12.09	**9.06**	8.48	8.74	**8.61**	250.39	489.42	**369.91**	80.10	-	**80.10**	-
S6 (V2)	15.08	-	**15.08**	7.21	10.37	**8.79**	13.44	14.96	**14.20**				-
S7	36.55	17.66	**27.11**	*		*	26.55	41.47	**34.01**	91.52	68.17	**79.85**	-
S8	23.35	17.76	**20.56**	17.04	-	**17.04**	58.45	39.98	**49.22**	53.04	143.16	**98.10**	-
													-
P35	12.06	13.35	**12.71**	8.98	13.84	**11.41**	131.47	-	**131.47**	20.34	8.84	**14.59**	-

Table 2: Foliar NH_4-N FW (µg g^{-1}) measured in the vegetation samples. '-' means no or not enough sample available. '*' means sample lost during sample processing

	S. capillifolium			*S. subnitens*			*Hypnum*			*Cladonia**			*Pleurozium*		
	frozen weight	dry weight	dry mass	frozen weight	dry weight	dry mass	frozen weight	dry weight	dry mass	frozen weight	dry weight	dry mass	frozen weight	dry weight	dry mass
site	(g)	(g)	%	(g)	(g)	%	(g)	(g)	%	(g)	(g)	%	(g)	(g)	%
C2	7.7456	1.1819	**15.3**	0.6937	0.0993	**14.3**	2.6206	0.6617	**25.2**	-	-	-	-	-	-
B2	2.0937	0.4907	**23.4**	4.7598	0.5198	**10.9**	3.1839	0.4976	**15.6**	-	-	-	-	-	-
V1	3.0445	0.4628	**15.2**	3.7018	0.4181	**11.3**	5.7873	0.9434	**16.3**	3.4089	0.6956	**20.4**	-	-	-
V2 (S6)															
V3	7.6661	0.8605	**11.2**	1.7830	0.2696	**15.1**	1.3192	0.4842	**36.7**	-	-	-	-	-	-
V4	6.3673	0.8933	**14.0**	2.5739	0.6961	**27.0**	2.4046	0.3828	**15.9**	-	-	-	-	-	-
V5	8.5424	1.2606	**14.8**	3.7057	0.5373	**14.5**	1.1969	0.4177	**34.9**	2.9527	1.6028	**54.3**	-	-	-
S1															
near S1	9.5499	1.4484	**15.2**	-	-	-	6.4095	1.2512	**19.5**	-	-	-	2.2929	0.6723	**29.3**
S2	9.7672	1.3419	**13.7**	5.4206	0.7222	**13.3**	1.8401	0.2873	**15.6**	3.7504	1.5958	**42.6**	-	-	-
S3	3.8256	0.5702	**14.9**	10.9847	1.0231	**9.3**	2.4289	0.5293	**21.8**	3.5258	1.0356	**29.4**	-	-	-
S4	12.9693	1.4395	**11.1**	7.3581	0.8033	**10.9**	2.2382	0.4415	**19.7**	1.9274	0.7793	**40.4**	-	-	-
S5	13.9799	1.4332	**10.3**	11.7988	1.3949	**11.8**	2.9940	0.5880	**19.6**	1.4678	0.8170	**55.7**	-	-	-
S6 (V2)	4.7183	0.7928	**16.8**	2.0035	0.3593	**17.9**	4.8835	0.7991	**16.4**						
S7	6.8162	0.7137	**10.5**	3.3690	0.3139	**9.3**	1.0011	0.2970	**29.7**	5.9283	1.9675	**33.2**	-	-	-
S8	19.0999	1.7826	**9.3**	10.5458	0.8442	**8.0**	6.1220	1.1072	**18.1**	2.6744	1.5942	**59.6**	-	-	-
P35	4.4699	0.7888	**17.6**	4.5070	0.4970	**11.0**	1.5195	0.3555	**23.4**	7.3418	3.5591	**48.5**	-	-	-

Table 3: Fresh and dry weight (g) and % dry mass from the left over vegetation samples. * *Cladonia* at V1 and V5 in bad condition. '-' means no or not enough sample available

NH4-N dry weight	*S. capillifolium*			*S. subnitens*			*Hypnum*			*Cladonia*			*Pleuroziur*
site\ NH4-N(µg g-1 DW)	A	B	mean	A	B	mean	A	B	mean	A	B	mean	A
C2	9.98	50.80	**30.39**	326.94	-	**326.94**	514.34	-	**514.34**	-	-	-	-
B2	117.93	-	**117.93**	112.60	134.61	**123.60**	1981.55	-	**1981.55**	-	-	-	-
V1	45.98	-	**45.98**	178.46	-	**178.46**	720.01	-	**720.01**	1067.98	-	**1067.98**	-
V2 (S6)													-
V3	267.80	219.16	**243.48**	47.72	48.91	**48.31**	160.45	-	**160.45**	-	-	-	-
V4	88.16	113.03	**100.60**	42.77	36.25	**39.51**	510.70	-	**510.70**	-	-	-	-
V5	39.44	32.51	**35.98**	43.54	108.82	**76.18**	130.70	-	**130.70**	23.68	39.87	**31.77**	-
S1	-	-	-	-	-	-	-	-	-	-	-	-	-
near S1	71.48	115.96	**93.72**	-	-	-	210.96	247.28	**229.12**	-	-	-	**77.64**
S2	84.71	-	**84.71**	85.74	80.63	**80.63**	279.80	-	**279.80**	80.84	553.79	**317.31**	-
S3	31.57	62.23	**46.90**	130.38	126.86	**128.62**	394.56	697.78	**546.17**	949.02	519.47	**734.24**	-
S4	162.06	202.45	**182.25**	287.02	-	**287.02**	2178.96	-	**2178.96**	719.51	-	**719.51**	-
S5	53.81	108.24	**81.03**	65.95	67.98	**66.96**	1181.34	2307.47	**1744.41**	137.92	-	**137.92**	-
S6 (V2)	82.74	61.70	**72.22**	37.15	53.49	**45.32**	75.75	84.46	**80.10**				
S7	320.42	154.84	**237.63**	*	-	*	83.72	130.74	**107.23**	259.02	192.80	**225.91**	-
S8	228.87	174.25	**201.56**	194.57	-	**194.57**	298.64	204.18	**251.41**	85.69	231.34	**158.51**	-
P35	63.21	69.97	**66.59**	74.83	115.33	**95.08**	520.89	-	**520.89**	39.96	17.37	**28.67**	-

Table 4: Foliar NH_4-N DW (µg g^{-1}) measured in the vegetation samples. '-' means no or not enough sample available. '*' means sample lost during sample processing.

APPENDIX IV: Ammonia air concentration (µg m^{-3}) from 2014-2019 for each monitoring site at Ballynahone Bog

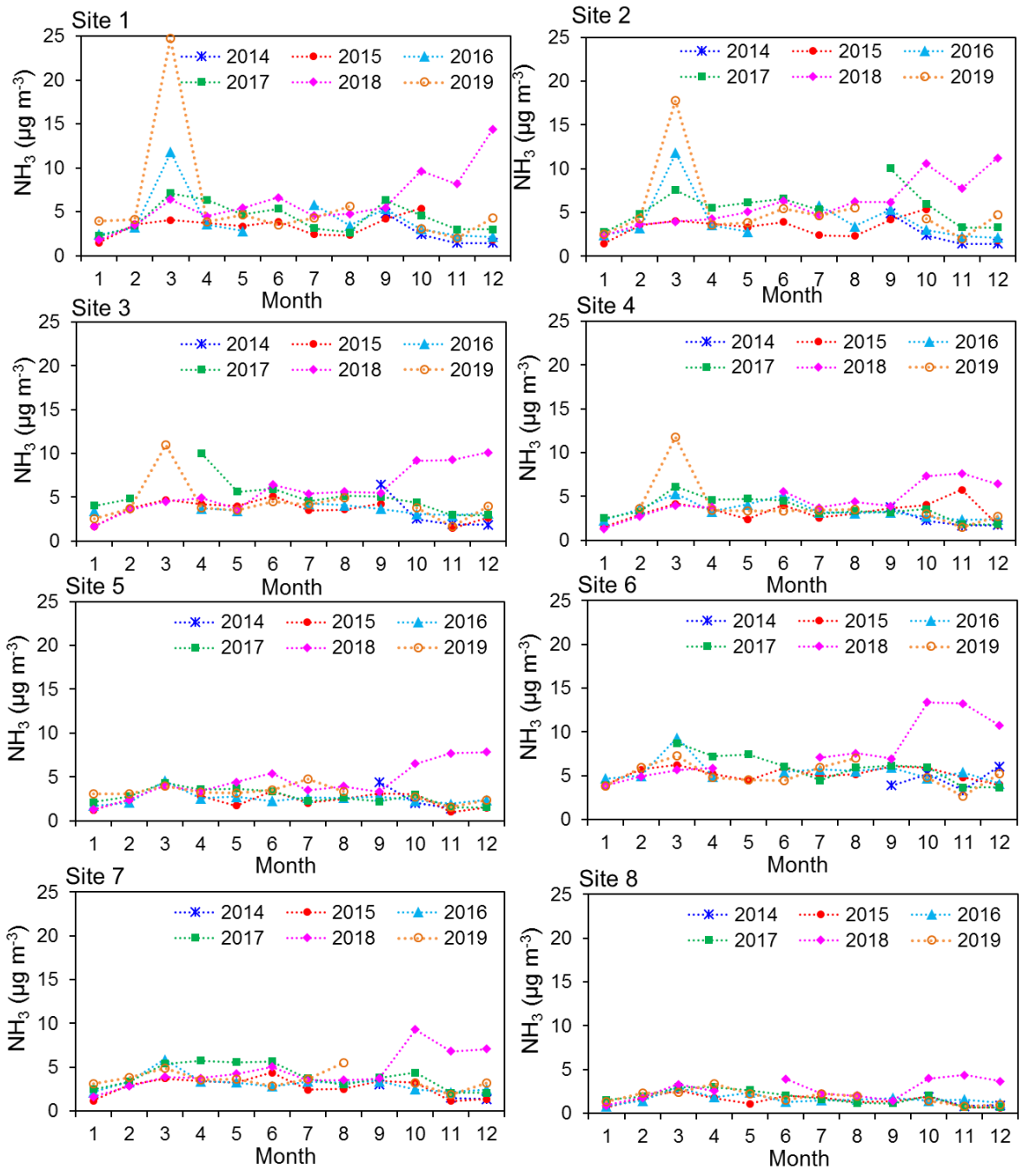


Figure 1: Monthly ammonia (NH_3) air concentration in µg m^{-3} at Ballynahone for the 8 monitoring sites over the years. Summary from Ballynahone Bog data report 2014-2019

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