UK Centre for Ecology & Hydrology

ICP VEGETATION: REVIEW OF NO_X CRITICAL LEVELS

MINUTES of the FIRST WORKSHOP

Workshop held online

Tuesday 24th May 2022

Workshop Background & Purpose

ICP Vegetation is responsible for reviewing and updating Chapter 3 of the Modelling and Mapping Manual, which considers critical levels for vegetation, and modelling and mapping critical level exceedances across Europe. The existing NO_x critical levels were first proposed in 1988 and set at an unchanged annual level (30 µg m^{-3}) since 1993. Therefore, it was deemed timely to review evidence around NO_x critical levels. As a first step in the review process, ICP Vegetation convened an online workshop of interested parties, advertised through ICP Vegetation channels (e.g. other ICP Task Force meetings), relevant conferences (e.g. Community of Air Pollution Effects Researchers) and by approaching corresponding authors of relevant literature following a brief review of recent publications.

The purpose of the workshop was to present the existing basis of current critical levels, give people the opportunity to present new research, to discuss findings, and to agree on an appropriate work plan to conduct a thorough review of NO_x critical levels. The latter was circulated among workshop participants following comments upon draft minutes.

Workshop Participants

In total, **37 people** were registered to attend the workshop, from **11 countries across Europe** (Austria, Czech Republic, Finland, Germany, Ireland, Italy, Portugal, Spain, Sweden, Switzerland, United Kingdom), with 3 attendees from the **United States of America** (from the Environmental Protection Agency and United States Forest Service).

Participants came from a **range of sectors**, including research scientists at university, non-governmental and governmental research organisations, policy-makers and policy-advisors from government and non-governmental agencies, and consulting firms.

Presented Topics

The agenda for the workshop can be found in Annex 1, while Annex 2 provides a list of submitted abstracts.

In brief, the workshop opened with presentations on the data background to the current levels (*Sabine Braun*) and a review of documents that underlie the Mapping Manual critical levels together with initial findings from a literature search (see Table of Literature section below) (*Mike Perring*). These talks both highlighted ambiguity in the current Mapping Manual text and underlying documentation (including suggestions that a relevant annual NO_x critical level is 15 µg m^{-3} rather than 30 µg m^{-3}), and the radically different atmospheric composition now as compared to when levels were first set.

Background talks were followed by a series of morning presentations on recent empirical work that together highlighted the range of research on NO_x air pollution effects on vegetation across boreal (*Sirkku Manninen*), temperate (*Elise Fox*) and Mediterranean (*Duncan Mifsud*) systems, including through many different techniques (e.g. survey of community composition dynamics, large-scale mapping, tree ring and isotope analysis). In the afternoon, there were three talks: one on lichen community dynamics in North America (*Tara Greaver*); one on tree pollen responses to short-term NO_x exposure (*Helena Ribeiro*); and, a third on recent work on NO_x and its relationship to dry deposition in Mediterranean systems (*Héctor Garcia-Gómez*).

Some additional slides were presented during the course of discussions. First, *Ben Marner* presented a useful analysis of how maximum daily air concentrations of NO_x related to annual mean values. *Cristina Branquinho* shared some relevant findings on lichen physiological and ecological response. Although all species responded negatively to increased NO_x concentrations, differences in the magnitude meant that the ecological endpoint (community change) led to some 'winners' and 'losers'. *Mark Sutton* considered how the pollutant mixture of NO_x with NH_3 can affect the setting of critical levels, in the light of lichen community responses to nitrogen air quality parameters.

These empirical talks revealed the breadth of recent work on air pollution effects on vegetation, and together with questions and discussions after talks, highlighted the complexity in determining drivers of vegetation change, and disentangling direct and indirect effects of nitrogen pollution (and thus relationships between critical levels and critical loads).

Summary of Discussion Topics

Below is a list of discussion topics considered in breakout groups and plenary discussion.

i) Do you agree with the definition of critical levels? If not, why not and how should it be changed?

ii) Is there a rationale(s) for making separate critical levels for NO and NO_2? If yes, what is it / are they? If no, why not?

iii) Do you know of evidence, not already discussed / presented, that would refine current NO_x critical levels? Please note e.g. whether the evidence is from fumigation studies, gradient studies and/or another approach; the timescale of application.

iv) What type of response can be considered adverse (i.e. what are indicators of effect) and how do we weigh the importance / reliability of evidence from different studies?

v) Is there a rationale(s) for retaining short-term ($\leq$ 1 day) critical levels, and/or long-term critical levels (i.e. annual average). Are other averaging periods important for risk to vegetation?

vi) What is the best method of calculation of critical level now, given available data? In comparison to what has happened in the past?

vii) Although we are concentrating on risks to vegetation, are you aware of research suggesting that other organisms / ecosystem components need considering in the light of air pollution? Could this have a bearing on NO_x critical levels more generally?

viii) Is there a rationale(s) for modifying NO_x critical levels for certain ecosystem types / vegetation groups?

ix) Is there a rationale(s) for modifying NO_x critical levels based on other pollutants / climate change / land use?

x) One of the uses of critical levels is to map exceedances. Are concentration-based metrics (as currently used for NO_x) the best way to map risk to vegetation? Do other methods need considering e.g. accumulation over threshold or flux-based measures like, for example, the phytotoxic ozone dose?

xi) What progress (if any) is needed to help map NO_x critical levels?

xii) In your opinion, what are the gaps (if any) in NO_x critical levels research?

xiii) In meetings considering NH_3 critical levels, there was some discussion about the relationship between critical loads and critical levels. Do you have any thoughts on how the critical level for NO_x (in whatever way it is refined) relates to critical loads?

Participants generally agreed that the definition of critical levels can be unchanged with one suggestion: whereas this is currently '*adverse effect on vegetation*', it was suggested to be changed to '*adverse effect on ecosystem functioning*' as this accounts for occurrences when there could be positive effects on an individual species, which have a knock-on negative effect more broadly. However, it was noted that it is also important that all terms in the definition are clearly defined themselves to avoid misinterpretation e.g. what is meant by direct effect *vs* indirect effect; how NO_x as the sum of NO and NO_2 is expressed as NO_2 equivalents.

There was an expectation that despite ongoing work on air pollution effects on vegetation, there will be limited additional available evidence to help revise the NO_x critical level(s), especially in the changed characteristics of the early- to mid-21st Century atmosphere. It was agreed that the literature that helped set the existing critical level should be re-evaluated to ascertain its reliability and robustness given changed environmental conditions since the early experimental work. It was agreed that use of additional metrics to establish and map critical levels and their exceedances should be considered. It will also be valuable to consider evidence for NO_x effects on other ecosystem components (e.g. pollinators), both in terms of direct impacts, but also through effects on vegetation e.g. decreased plant fitness due to impaired pollination.

Next Steps and Proposed Timeline

Working groups will be established, with the following possible topics:

- Analysis of spatial and temporal consistency in maximum daily mean vs annual mean NO_x plus more general mapping of air pollution context (historical, current and expected) in Europe.

- Evidence/data extraction from literature search.

- Background Document text. Anticipated sections are:

  - Separate effects of NO and NO_2 on vegetation and processes by which they affect vegetation and recommendations for further research.
  - Evidence for NO_x effects on other ecosystem components.
  - Explanation of all terms in critical level definition, including calculation of NO_2 equivalence, what constitutes an adverse effect, etc; also consider short- vs long-term critical levels.

- Relationship(s) between critical levels and critical loads.
- Rationale and/or evidence for modification of critical levels based on other air pollutants / climate change / land use / vegetation types / ecosystem types.
- Alternative critical level calculation methods (cf. Cape et al. 2009).
- Comparison of how spatial critical level exceedance varies with different metrics of risk to vegetation.
- Metrics to best represent risk to vegetation.

The anticipated timeline for the review process is:

May 24th 2022:	First online workshop on ICP Vegetation Review of NO_x Critical Levels.
June 9th 2022:	Request **for volunteers for different workgroups.** To respond by 16th June 2022.
June 24th 2022:	Finalised minutes and report from Workshop 1 circulated to participants, and published on ICP Vegetation website soon thereafter.
Mid-July 2022:	**Finalise participation in working groups**
Mid-July to mid-Oct 2022:	Working groups progress evidence extraction, analysis and text development. Submit findings to ICP Vegetation Programme Centre mid-October 2022.
Mid-Nov 2022:	Second online workshop to address questions / issues / matters arising.
Mid-Nov 2022 to mid-Jan 2023:	Working groups convene to address developments from second online workshop.
Mid-Jan 2023:	Working groups submit recommendations to ICP Vegetation Programme Centre.
20th – 23rd Feb 2023:	**ICP Vegetation Task Force Meeting –** seek agreement on revised Mapping Manual text and Background Document. Feed up to WGE and LRTAP as appropriate.

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Table of Literature found during systematic searches / via expert feedback, March 2022 [*correct as of 24th June 2022*]

Author(s)	**Year**	**Title**
Ammann et al.	1999	*Estimating the uptake of traffic-derived NO2 from N-15 abundance in Norway spruce needles*
Angold	1997	*The impact of a road upon adjacent heathland vegetation: Effects on plant species composition*
Assersohn	2022	*A rapid evidence review of the impacts of air pollution on terrestrial invertebrates* [not publically available – inspect reference list within]
Banerjee et al.	2021	*Variation of tree biochemical and physiological characters under different air pollution stresses*
Bates et al.	2001	*Loss of Lecanora conizaeoides and other fluctuations of epiphytes on oak in SE England over 21 years with declining SO2 concentrations*
Bell et al.	2011	*Effects of vehicle exhaust emissions on urban wild plant species*
Bignal et al.	2008	*Effects of air pollution from road transport on growth and physiology of six transplanted bryophyte species*
Bosela et al.	2014	*Possible causes of the recent rapid increase in the radial increment of silver fir in the Western Carpathians*
Breuninger et al.	2013	*Field investigations of nitrogen dioxide (NO_2) exchange between plants and the atmosphere*
Campbell and Vallano	2018	*Plant defences mediate interactions between herbivory and the direct foliar uptake of atmospheric reactive nitrogen.*
Chaparro-Suarez et al.	2011	*Nitrogen dioxide (NO2) uptake by vegetation controlled by atmospheric concentrations and plant stomatal aperture.*
Chen et al.	2019	*Characteristics and influence factors of NO2 exchange flux between the atmosphere and* P. nigra*.*
Contardo et al.	2021	*Biological Effects of Air Pollution on Sensitive Bioindicators: A Case Study from Milan, Italy*
Davies et al.	2007	*Diversity and sensitivity of epiphytes to oxides of nitrogen in London*
Delaria et al.	2018	*Measurements of NO and NO2 exchange between the atmosphere and* Quercus agrifolia*.*
Delaria et al.	2020	*Laboratory measurements of stomatal NO2 deposition to native California trees and the role of forests in the NOx cycle.*
Egerton-Warburton et al.	2001	*Reconstruction of the historical changes in mycorrhizal fungal communities under anthropogenic nitrogen deposition*
Fenn et al.	2007	*Atmospheric deposition inputs and effects on lichen chemistry and indicator species in the Columbia River Gorge, USA*
Field et al.	2014	*The role of nitrogen deposition in widespread plant community change across semi-natural habitats*
Fowler et al.	1998	*The atmospheric budget of oxidized nitrogen and its role in ozone formation and deposition.*
Frati et al.	2006	*Effects of NO(2) and NH(3) from road traffic on epiphytic lichens*
Gadsdon and Power	2009	*Quantifying local traffic contributions to NO2 and NH3 concentrations in natural habitats*
Garcia-Gomez et al.	2016	*Atmospheric pollutants in peri-urban forests of Quercus ilex: evidence of pollution abatement and threats for vegetation*

Author(s)	Year	Title
Gessler et al.	2000	*NH3 and NO2 fluxes between beech trees and the atmosphere–correlation with climatic and physiological parameters.*
Hajeck et al.	2021	*Effect of Climate Change on the Growth of Endangered Scree Forests in Krkonose National Park (Czech Republic)*
Hanson and Lindberg	1991	*Dry deposition of reactive nitrogen compounds: a review of leaf, canopy and non-foliar measurements.*
Hargreaves et al.	1992	*The exchange of nitric oxide, nitrogen dioxide and ozone between pasture and the atmosphere.*
Hazewinkel et al.	2008	*Have atmospheric emissions from the Athabasca Oil Sands impacted lakes in northeastern Alberta, Canada?*
Honour et al.	2009	*Responses of herbaceous plants to urban air pollution: Effects on growth, phenology and leaf surface characteristics*
Hu et al.	2015	*Gaseous NO_2 effects on stomatal behaviour, photosynthesis and respiration of hybrid poplar leaves*
Huang et al.	2021	*Significant contributions of combustion-related NH3 and non-fossil fuel NOx to elevation of nitrogen deposition in southwestern China over past five decades*
Hultengren et al.	2004	*Recovery of the epiphytic lichen flora following air quality improvement in south-west Sweden*
Iodice et al.	2016	*Air pollution monitoring using emission inventories combined with the moss bag approach*
Ishii et al.	2007	*Phytotoxic risk assessment of ambient air pollution on agricultural crops in Selangor State, Malaysia*
Jenkins et al.	2021	*Air Pollution and Climate Drive Annual Growth in Ponderosa Pine Trees in Southern California*
Jochner et al.	2015	*The effects of short- and long-term air pollutants on plant phenology and leaf characteristics*
Jovan et al.	2012	*Eutrophic lichens respond to multiple forms of N: implications for critical levels and critical loads research*
Jovan & McCune	2005	*Air-quality bioindication in the greater central valley of California, with epiphytic macrolichen communities*
Kirkham et al.	2001	*Nitrogen uptake and nutrient limitation in six hill moorland species in relation to atmospheric nitrogen deposition in England and Wales*
Klap et al.	2000	*Effects of environmental stress on forest crown condition in Europe. Part IV: Statistical analysis of relationships*
Kralicek et al.	2017	*Dynamics and structure of mountain autochthonous spruce-beech forests: impact of hilltop phenomenon, air pollutants and climate*
Krzyzaniak et al.	2021	*Factors Influencing the Health Status of Trees in Parks and Forests of Urbanized Areas*
Kupcinskiene	2001	*Annual variations of needle surface characteristics of Pinus sylvestris growing near the emission source*
Larsen et al.	2007	*Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity*
Laxen and Marner	2008	*NO_2 concentrations and distance from roads [Report for Defra by Air Quality Consultants]*
Laxen, Marner and Donovan	2007	*Deriving NO_2 from NO_x for air quality assessments of roads – updated to 2006 [Report for Defra by Air Quality Consultants]*
Laxton et al.	2010	*An assessment of nitrogen saturation in Pinus banksiana plots in the Athabasca Oil Sands Region, Alberta*
Lerdau et al.	2000	*The NO2 flux conundrum.*
Manai et al.[†]	2014	*Exogenous nitric oxide (NO) ameliorates salinity-induced oxidative stress in tomato (Solanum lycopersicum) plants*

Author(s)	Year	Title
Manninen and Huttunen	2000	*Response of needle sulphur and nitrogen concentrations of Scots pine versus Norway spruce to SO_2 and NO_2*
Manninen	2018	*Deriving nitrogen critical levels and loads based on the responses of acidophytic lichen communities on boreal urban* Pinus sylvestris *trunks*
Mathias & Thomas	2018	*Disentangling the effects of acidic air pollution, atmospheric CO2, and climate change on recent growth of red spruce trees in the Central Appalachian Mountains*
Mattei et al.	2022	*Traffic-related NO_2 affects expression of* Cupressus sempervirens *L. pollen allergens*
Mayer et al.	2013	*Significant decrease in epiphytic lichen diversity in a remote area in the European Alps, Austria*
Mifsud et al.	2021	*A preliminary study into the use of tree-ring and foliar geochemistry as bio-indicators for vehicular NOx pollution in Malta*
Modrzynski et al.	2003	*Defoliation of older Norway spruce (Picea abies L Karst.) stands in the Polish Sudety and Carpathian mountains*
Morikawa et al.	1998	*More than a 600-fold variation in nitrogen dioxide assimilation among 217 plant taxa*
Muller et al.	1996	*Interaction between atmospheric and pedospheric nitrogen nutrition in spruce (Picea abies L Karst) seedlings*
Nash	1976	*Sensitivity of lichens to nitrogen dioxide fumigations*
Palmer et al.	2004	*Biodiversity in roadside verges: Final Report*
Pasqualini et al.	2003	*Phenolic compounds content in Pinus halepensis Mill. needles: a bioindicator of air pollution*
Payne et al.	2013	*Impact of nitrogen deposition at the species level*
Pereira et al.	2021	*The Strong and the Stronger: The Effects of Increasing Ozone and Nitrogen Dioxide Concentrations in Pollen of Different Forest Species*
Pilegaard et al.	1998	*Fluxes of ozone and nitrogen dioxide measured by Eddy correlation over a harvested wheat field.*
Redling et al.	2013	*Highway contributions to reactive nitrogen deposition: tracing the fate of vehicular NOx using stable isotopes and plant biomonitors*
Rogerieux et al.	2007	*Modifications of Phleum pratense grass pollen allergens following artificial exposure to gaseous air pollutants (O-3, NO2, SO2)*
Saurer et al.	2004	*First detection of nitrogen from NOx in tree rings: a N-15/N-14 study near a motorway*
Sénéchal et al.	2015	*A review of the effects of major atmospheric pollutants on pollen grains, pollen content, and allergenicity*
Singh et al.	2021	*Tree responses to foliar dust deposition and gradient of air pollution around opencast coal mines of Jharia coalfield, India: gas exchange, antioxidative potential and tolerance level*
Smith et al.	2020	*Epiphytic macrolichen communities indicate climate and air quality in the US Midwest*
Sparks	2009	*Ecological ramifications of the direct foliar uptake of nitrogen*
Sun et al.	2020	*Arbuscular mycorrhizal fungus-mediated amelioration of NO2-induced phytotoxicity in tomato*
Sutton	2019	*Risks from air pollution to the integrity of Ashdown Forest Special Area of Conservation: Overview of Issues and Conclusions*
Takahashi et al.	2005	*Differential assimilation of nitrogen dioxide by 70 taxa of roadside trees at an urban pollution level*

Author(s)	Year	Title
Takahashi and Morikawa	2014	*Nitrogen dioxide is a positive regulator of plant growth.*
Takahashi et al.	2014	*Nitrogen dioxide regulates organ growth by controlling cell proliferation and enlargement in Arabidopsis.*
Teklemariam and Sparks	2006	*Leaf fluxes of NO and NO2 in four herbaceous plant species: the role of ascorbic acid.*
Thoene et al.	1996	*Absorption of atmospheric NO2 by spruce (*Picea abies*) trees: II. Parameterization of NO2 fluxes by controlled dynamic chamber experiments.*
Truscott et al.	2005	*Vegetation composition of roadside verges in Scotland: the effects of nitrogen deposition, disturbance and management*
Vacek et al.	2019	*Adaption of Norway spruce and European beech forests under climate change: from resistance to close-to-nature silviculture*
Vandinther	2019	*The influence of nitrogen deposition on community composition in* Pinus banksiana *forests across north western Canada. MSc Thesis, Trent University, Ontario*
Wang et al.	2021	*Atmospheric nitrogen dioxide at different concentrations levels regulates growth and photosynthesis of tobacco plants*
Weber and Rennenberg	1996	*Dependency of nitrogen dioxide (NO2) fluxes to wheat (*Triticum aestivum *L.) leaves from NO2 concentration, light intensity, temperature and relative humidity determined from controlled dynamic chamber experiments.*
Wesely	1989	*Parameterization of surface resistances to gaseous dry deposition in regional-scale numerical models.*
Wesely and Hicks	2000	*A review of the current status of knowledge on dry deposition.*
Wesely et al.	1982	*An eddy-correlation measurement of NO2 flux to vegetation and comparison to O3 flux.*
Wilkins et al.	2016	*Vegetation community change points suggest that critical loads of nutrient nitrogen may be too high*
Wolseley et al.	2014	*Guide to using a lichen based index to nitrogen air quality: Field Studies Council*
Zhao et al.	2021	*Effects of air pollution on physiological traits of Ligustrum lucidum Ait. leaves in Luoyang, China*

[†]: Inserted as example of NO for stress tolerance. Need to check original paper as to whether actually applied as NO or as another substance that can generate NO. See also e.g. Gadelha et al. 2017 *Exogenous nitric oxide improves salt tolerance during establishment of Jatropha curcas seedlings by ameliorating oxidative damage and toxic ion accumulation*

Egham Report References

The following references were contained within Caporn (1993) Critical levels for NO_2 pp. 48 – 54 in Critical Levels of Air Pollutants for Europe (Ashmore and Wilson (Eds.))

Author(s)	Year	Title
Ashenden et al.	1990	*Effects of nitrogen dioxide on the growth of three fern species*
Atkinson et al.	1991	*Atmospheric pollution and the sensitivity of stomata on barley leaves to abscisic acid and carbon dioxide.*

Author(s)	**Year**	**Title**
Bell et al.	1992	*Effects of rural roadside levels of nitrogen dioxide on* Polytrichum formosum *Hedw.*
Bennett et al.	1990	*Inhibition of photosynthesis and leaf conductance interactions induced by SO_2, NO_2 and SO_2 + NO_2*
Besford and Hand	1989	*The effects of CO_2 enrichment and nitrogen oxides on some calvin cycle enzymes and nitrite reductase in glasshouse lettuce*
Caporn	1989	*The effects of oxides of nitrogen and CO_2 enrichment on photosynthesis and growth of lettuce (*Lactuca sativa *L.)*
Caporn et al.	1991	*NO_2 plus SO_2 stimulates growth but reduces frost tolerance in* Calluna vulgaris [*In abstracts of NERC CAPER meeting 1991*]
Caporn et al.	1991	*Low temperature-enhanced inhibition of photosynthesis by oxides of nitrogen in lettuce (*Lactuca sativa *L.)*
Fowler et al.	1988	*Effects of air filtration at small SO_2 and NO_2 concentrations on the yield of barley*
Freer-Smith	1984	*The responses of six broadleaved trees during long term exposure to SO_2 and NO_2*
Guderian	1988	*Critical levels for effects of NO_2 [Working paper for critical levels workshop Bad Harzburg, 1988] Final draft report of ECE Critical Levels Workshop, Bad Harzburg, 14–18 March 1988. Geneva, United Nations Economic Commission for Europe, 1988, pp. 79–104.*
Hanson and Lindberg	1991	*Dry deposition of reactive nitrogen compounds: a review of leaf canopy and non-foliar measurements*
Hill and Bennet	1970	*Inhibition of apparent photosynthesis by nitrogen oxides*
Klumpp et al.	1989	*Nitrate reductase activity of needles of Norway Spruce fumigated with different mixtures of O_3, SO_2 and NO_2*
Lendzian and Kerstiens	1988	*Interactions between plant cuticles and gaseous air pollutants*
Lucas	1990	*The effects of prior exposure to SO_2 and NO_2 on the water relations of Timothy grass (*Phleum pratense*) under drought conditions*
Morgan et al.	1992	*Effects of nitrogen oxides on nitrate assimilation in bryophytes*
Mortensen	1986	*Nitrogen oxides produced CO_2 enrichment III. Effects on tomato at different photon flux densities*
Näsholm et al.	1991	*Uptake of NO_x by mycorrhizal and non-mycorrhizal Scots pine seedlings: quantities and effects on amino acid and protein concentrations*
Neighbour et al.	1990	*Purafil-filtration prevents the development of ozone-induced frost injury: a potential role for nitric oxide*
Norby et al.	1989	*Induction of nitrate reductase activity in red spruce needles by NO_2 and HNO_3 vapour.*
Okano and Totsuka	1986	*Absorption of NO_2 by sunflower plants grown at various levels of nitrate*
Rowland et al.	1987	*Foliar entry and incorporation of atmospheric nitrogen dioxide into barley plants of different nitrogen status*
Rowland-Bamford et al.	1989	*NO_2 flux into leaves of nitrate reductase deficient barley mutants and corresponding changes in nitrate reductase activity*
Sabaratnum and Gupta	1988	*Effects of NO_2 on biochemical and physiological characteristics of soyabean*

Author(s)	Year	Title
Sandhu and Gupta	1989	*Effects of NO_2 on growth and yield of black turtle bean (*Phaseolus vulgaris *L.) cv Domino*
Saxe	1987	*Stomate-dependent and stomate-independent uptake of NO_x and effects on photosynthesis, respiration and transpiration of potted plants. In: Effects of atmospheric pollutants on forests, wetlands and agricultural ecosystems (Hutchinson and Meema eds.)*
Taylor and Bell	1988	*Studies on the tolerance to SO_2 of grass populations in polluted areas V. Investigations into the development of tolerance to SO_2 and NO_2 in combination and NO_2 alone.*
Thoene et al.	1991	*Absorption of atmospheric NO_2 by spruce (*Picea abies *L. Karst.) trees I. NO_2 influx and its correlation with nitrate reduction*
Wellburn	1990	*Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers?*
Whitmore and Freer-Smith	1982	*Growth effects of SO_2 and/or NO_2 on woody plants and grasses during spring and summer*
Wingsle et al.	1987	*Induction of nitrate reductase in needles of Scots pine seedlings by NO_x and NO_3^-*
Wolfenden et al.	1991	*Effects of over-wintering fumigation with sulphur and nitrogen dioxides on biochemical parameters and spring growth in red spruce (*Picea rubens*)*
Wright	1987	*Effects of SO_2 and NO_2, singly and in mixtures, on the macroscopic growth of three Birch clones*

References contained within Tables 2, 3 and 5 of Chapter 11 of the Air Quality Guidelines – Second Edition

In grey if already included in Egham Report.

Author(s)	Year	Title
Adaros et al.	1991	*Concurrent exposure to SO2 alters the growth and yield responses of wheat and barley to low concentrations of CO2.*
Adaros et al.	1991	*Single and interactive effects of low levels of O3, SO2 and NO2 on the growth and yield of spring rape.*
Anderson and Mansfield	1979	*The effects of nitric oxide pollution on the growth of tomato.*
Ashenden et al.	1993	*Critical loads of N & S deposition to semi-natural vegetation. Bangor, Institute for Terrestrial Ecology, 1993 (Report proj. T07064L5).*
Ashenden	1979	*Effects of SO2 and NO2 pollution on transpiration in* Phaseolus vulgaris *L.*
Ashenden et al.	1990	*Effects of nitrogen dioxide pollution on the growth of three fern species.*
Bell et al.	1992	*Effects of rural roadside levels of nitrogen dioxide on* Polytrichum formosum.
Bender et al.	1991	*Response of nitrogen metabolism in bean (*Phaseolus vulgaris*) after exposure to ozone and nitrogen dioxide, alone and in sequence.*
Bennett et al.	1990	*Inhibition of photosynthesis and leaf conductance interactions induced by SO2, NO2 and SO2 + NO2.*

Author(s)	Year	Title
Besford and Hand	1989	*The effects of CO_2 enrichment and nitrogen oxides on some Calvin cycle enzymes and nitrite reductase in glasshouse lettuce.*
Bruggink et al.	1988	*The effect of nitric oxide fumigation at two CO_2 concentrations on net photosynthesis and stomatal resistance of tomato (*Lycopersicon lycopersicum *L. cv. Abunda).*
Bush et al.	1962	*The effects of engine exhaust on the atmosphere when automobiles are equipped with afterburners. Los Angeles, CA, University of California, 1962 (Report 62-63).*
Caporn	1989	*The effects of oxides of nitrogen and carbon dioxide enrichment on photosynthesis and growth of lettuce* (Lactuca sativa *L.).*
Caporn and Mansfield	1976	*Inhibition of net photosynthesis in tomato in air polluted with NO and NO_2.*
Caporn et al.	1991	*Low-temperature-enhanced inhibition of photosynthesis by oxides of nitrogen in lettuce* (Lactuca sativa *L.).*
Carlson	1993	*Interaction between SO_2 and NO_2 and their effects on photosynthetic properties of soybean* Glycine max.
Davison et al.	1987	*Interactions between air pollutants and cold stress. In: Schulte-Hostede, S. et al., ed. Proceedings of the 2nd International Symposium on Air Pollution & Plant Metabolism, Munich, 1987, pp. 307–328.*
Freer-Smith	1984	*The responses of six broadleaved trees during long term exposure to SO_2 and NO_2.*
Goodyear and Ormrod	1988	*Tomato response to concurrent and sequential NO_2 and O_3 exposures.*
Hill and Bennet	1970	*Inhibition of apparent photosynthesis by nitrogen oxides.*
Houlden et al.	1990	*Air pollution and agricultural aphid pests. I. Fumigation experiments with SO_2 and NO_2*
Hur and Wellburn	1994	*Effects of atmospheric NO_2 on Azolla–Anabaena symbiosis.*
Ito et al.	1984	*Effects of NO_2 and O_3 alone or in combination on kidney bean plants. II. Amino acid pool size and composition.*
Lane and Bell	1984	*The effects of simulated urban air pollution on grass yield. Part 2. Performance of* Lolium perenne, Phleum pratense *and* Dactylus glomerata *fumigated with SO_2, NO_2 and/or NO.*
Mehlhorn and Wellburn	1987	*Stress ethylene formation determines plant sensitivity to ozone.*
Mooi	1984	*Wirkungen von SO_2, NO_2, O_3 und ihre Mischungewn auf Pappeln und andere [Pflanzenarten. Forst- und Holzwirt, 39: 438–444]*
Morgan et al.	1992	*Effects of nitrogen oxides on nitrate assimilation in bryophytes*
Murray et al.	1994	*Effects of SO_2 and NO_2 on growth and nitrogen concentrations in lucerne and barrel medic.*
Murray et al.	1992	*Effects of NO_2 on hoop pine can be counteracted by SO_2.*
Näsholm et al.	1991	*Uptake of NOx by mycorrhizal and non-mycorrhizal Scots pine seedlings: quantities and effects on amino acid and protein concentrations.*
Neighbour et al.	1988	*Effects of sulphur dioxide and nitrogen dioxide on the control of water loss by birch* (Betula *spp.).*

Author(s)	Year	Title
Norby et al.	1989	*Induction of nitrate reductase activity in red spruce needles by NO2 and HNO3 vapor.*
Okano et al.	1985	*Growth responses of plants to various concentrations of nitrogen dioxide.*
Petitte and Ormrod	1992	*Sulfur dioxide and nitrogen dioxide affect growth, gas exchange and water relations of potato plants.*
Runeckles and Palmer	1987	*Pretreatment with nitrogen dioxide modifies plant response to ozone –short communication.*
Sabarathnam et al.	1988	*Effects of nitrogen dioxide on leaf chlorophyll and nitrogen content of soybean.*
Sabarathnam et al.	1988	*Nitrogen dioxide effects on photosynthesis in soyabean.*
Sandhu and Gupta	1989	*Effects of nitrogen dioxide on growth and yield of black turtle bean (*Phaseolus vulgaris *L.) cv. Domino.*
Saxe	1986	*Effects of NO2 and CO2 on net photosynthesis, dark respiration and transpiration of pot plants.*
Taylor and Eaton	1966	*Suppression of plant growth by NO2.*
Thoene et al.	1991	*Absorption of atmospheric NO2 by spruce (*Picea abies*) trees. I. NO2 influx and its correlation with nitrate reduction.*
Thompson et al.	1970	*Effects of ambient levels of NO2 on navel oranges.*
Van Hove et al.	1992	*Physiological effects of a long term exposure to low concentrations of NH3, NO2 and SO2 on Douglas fir (*Pseudotsuga menziesii*).*
Van der Eerden and Duym	1988	*An evaluation method for combined effects of SO2 and NO2 on vegetation*
Wellburn et al.	1981	*Biochemical explanation of more than additive inhibitory low atmospheric levels of SO2 + NO2 upon plants.*
Whitmore	1985	*Relationship between dose of SO2 and NO2 mixtures and growth of* Poa pratensis
Whitmore and Freer-Smith	1982	*Growth effects of SO2 and/or NO2 on woody plants and grasses during spring and summer.*
Yang et al.	1983	*Effects of pollutant combinations at low doses on growth of forest trees.*
Yoneyama et al.	1979	*Absorption of atmospheric NO2 by plants and soil. II. Nitrite accumulation, nitrite reductase activity and diurnal change of NO2 absorption in leaves.*

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Other Possibly Relevant References in list from Chapter 11 of the Air Quality Guidelines – Second Edition, not otherwise included in tables above

Author(s)	Year	Title
Benedict and Breen	1955	*The use of weeds as a means of evaluating vegetation damage caused by air pollution. In: Proceedings of the 3rd National Air Pollution Symposium, Los Angeles, 1955, pp. 177–190.*
Bücker and Ballach	1992	*Alterations in carbohydrate levels in leaves of* Populus *due to ambient air pollution.*
Cape	1993	*Direct damage to vegetation caused by acid rain and polluted cloud: definition of critical levels for forest trees*
Caporn et al.	1994	*Canopy photosynthesis of CO2-enriched lettuce (*Lactuca sativa *L.). Response to short-term changes in CO2, temperature and oxides of nitrogen.*
Flücker and Braun	1986	*Effects of air pollutants on insects and host/insect relationships. In: Proceedings of a Workshop jointly organised by the Commission of the European Communities and the National Agency for Environmental Protection, Risø, Denmark, March 1986. Brussels, European Commission, 1986.*
Fowler et al.	1980	*The influence of a polluted atmosphere on outside degradation in Scots pine (*Pinus sylvestris*). In: Drabløs, D. & Tollan, A., ed. Proceedings of the International Conference on the Ecological Impact of Acid Precipitation, Sandefjord, Norway, 1980. Oslo, Ås, 1980, pp. 156–157.*
Grennfelt et al.	1983	*Deposition and uptake of atmospheric nitrogen oxides in a forest ecosystem.*
Kosta-Rick and Manning	1993	*Radish (*Raphanus sativus *L.): a model for studying plant responses to air pollutants and other environmental stresses.*
Kuppers and Klump	1988	*Effects of ozone, sulfur dioxide, and nitrogen dioxide on gas exchange and starch economy in Norway spruce (*Picea abies *[L.] Karsten).*
Lee et al.	1993	*Sphagnum species and polluted environments, past and future.*
Mortensen	1985	*Nitrogen oxides produced during CO2 enrichment. II. Effects on different tomato and lettuce cultivars.*
Pande and Mansfield	1985	*Responses of spring barley to SO2 and NO2 pollution.*
Press et al.	1986	*The potential importance of an increased atmospheric nitrogen supply to the growth of ombrotrophic* Sphagnum *species.*
Raven	1988	*Acquisition of nitrogen by the shoots of land plants: its occurrence and implications for acid–base regulation.*
Saxe	1994	*Relative sensitivity of greenhouse pot plants to long-term exposures of NO and NO2-containing air.*
Saxe and Voight Christensen	1984	*Effects of carbon dioxide with and without nitric oxide pollution on growth, morphogenesis and production time of potted plants.*

Author(s)	Year	Title
Schulze et al.	1989	*Forest decline and air pollution. Berlin, Springer Verlag (Ecological Studies No. 77)*
Srivastava and Ormrod	1986	*Effects of nitrogen dioxide and nitrate nutrition on nodulation, nitrogenase activity, growth and nitrogen content of bean plants.*
Srivastava et al.	1975	*The effects of environmental conditions on the inhibition of leaf gas exchange by NO2.*
Steubing et al.	1989	*Effects of SO2, NO2 and O3 on population development and morphological and physiological parameters of native herb layer species in a beech forest.*
Taylor et al.	1987	*Air pollution injury to vegetation. London, IEHO.*
Van der Eerden et al.	1994	*Influence of nitrogenous air pollutants on carbon dioxide and ozone effects on vegetation. In: Jackson, M. & Black, C.R., ed. Interacting stresses on plants in a changing climate. Heidelberg, Springer, 1994, pp. 125–137.*
Zierock et al.	1986	*Studies on the need of a NO2 long term limit value for the protection of terrestrial and aquatic ecosystems. Luxembourg, Office for Official Publications of the European Communities, 1986 (CEC Final Report EUR 10 546 EN).*

Annex 1

AGENDA

Conceptual Background and Empirical Investigations I **(Chair: Felicity Hayes, UKCEH)**

09.15: Welcome and Plan for the Day (Mike Perring, UKCEH)

09.30: The data basis of the current critical levels (Sabine Braun, Institute for Applied Plant Biology)

09.50: A brief review of current literature (Mike Perring, UKCEH)

10.10: Discussion Time / Questions Arising

10.30: COFFEE BREAK

Empirical Investigations II **(Chair: Mike Perring, UKCEH)**

11.00: The structure of epiphytic macrolichen community in relation to modelled NO_2 concentration in a boreal city (Sirkku Manninen, University of Helsinki)

11.20: The Influence of NOx Emissions on the Nitrogen Isotopic Composition of Tree Rings and Foliage (Duncan Mifsud, University of Kent)

11.40: Challenges in modelling the effects of NOx concentrations on UK bryophyte distribution (Elise Fox, Liverpool John Moore's University)

12.00 – 12.30: First breakout discussion. Please see breakout discussion questions.

12.30 – 12.45: Feedback, in plenary, on first breakout discussions (Chair: Felicity Hayes, UKCEH)

12.45 – 13.30: LUNCH

Empirical Investigations III **(Chair: Katrina Sharps, UKCEH)**

13.30: Effects of increasing nitrogen dioxide concentrations in pollen of different forest species (Helena Ribeiro, University of Porto)

13.50: Synthesis of published lichen response to gaseous nitrogen: ammonia versus nitrogen dioxide (Tara Greaver, US EPA)

14.10: NO2 measurements in Spanish Mediterranean forests in the context of dry deposition (Héctor García-Gómez, CIEMAT)

14.30: COFFEE BREAK

14.55: **Introduction to second breakout discussions** (Mike Perring, UKCEH)

15.00 – 15.40: **Second breakout discussion. Please see breakout discussion questions.**

15.40 – 16.00: **Feedback, in plenary, from second breakout discussions (Chair: Mike Perring, UKCEH)**

16.00: **Next steps** (Mike Perring, UKCEH)

16.20: **Meeting close**

Annex 2

SUBMITTED ABSTRACTS

Sabine Braun, Institute for Applied Plant Biology

The data basis of the current critical levels

This talk will highlight how stakeholders arrived at the existing NOx critical levels for vegetation. It will also present some reflections on earlier debates around the setting of critical levels, especially in the light of interactions with other pollutants, and links to subsequent work on N critical loads. The talk is presented by one of the original participants in the Egham (1992) workshop

Elise Fox, Liverpool John Moore's University; Hayes, F., UKCEH; Dalrymple, S., Liverpool John Moore's University

Challenges in modelling the effects of NOx concentrations on UK bryophyte distribution

Previous critical levels for semi-natural vegetation have been informed from data regarding vascular plants however, mosses, hornworts and liverworts have been neglected in these calculations. This has been mainly due to challenges in quantifying pollutant fluxes at fine scale. These fluxes are largely governed by boundary layer processes that are difficult to measure. Bryophytes help regulate nutrient supply in plant communities and are a microhabitat for many other organisms and therefore, should be accounted for when reviewing NOx critical levels.

Héctor García-Gómez, CIEMAT

NO2 measurements in Spanish Mediterranean forests in the context of dry deposition

During 2016-2017, four holm oak forests were intensively monitored (meteorology, soil water content, nitrogen deposition, gaseous pollutants with passive and active samplers, particulate nitrogen, etc.) in Spain. Below-canopy concentrations of N gaseous pollutants were significant smaller than levels found in the open field. For NO2, those reductions (up to 41%) were comparable to, and even higher than, values reported in similar empirical studies with deciduous forest species. This evidence of air quality improvement for Quercus ilex forests requires specifically designed monitoring programs of urban and peri-urban forests to quantify the relevance of this ecosystem service and understand the environmental processes involved. Stomatal uptake of NH3, HNO3 and NO2 derived from the DO3SE (Deposition of Ozone and Stomatal Exchange) model, was estimated to calculate total dry deposition of inorganic N air pollutants in these four forests. The stomatal deposition of N gases averaged for the four sites 3.3 ± 0.8 kg N ha-1 year-1, with NO2 contributing the most (2.0 ± 0.4 kg N ha-1 year-1), contributing deposition averaged from 19% in the peri-urban forests to 11% in the most natural site.

Tara Greaver, US EPA

Synthesis of published lichen response to gaseous nitrogen: ammonia versus nitrogen dioxide

In this synthesis, we characterize U.S. air concentrations of the most ubiquitous gaseous forms of oxidized nitrogen, NO2, and its direct effects on lichens. In the U.S., the 3-year average (2017-2019) of the annual mean for each monitoring site ranges up to 30 ppb (~56.4 ug m -3) for NO2. The spatial coverage of current routine monitoring of NO2 likely does not accurately represent exposures of NO2 to ecosystems in rural areas. NH3 can act as a nutrient to lichens, but as exposure rise, both can cause physiological stress, and mortality. There is a growing body of evidence that lichen community composition is altered at current levels of exposure in the U.S., with no effect concentrations from <1-3 ug m -3 NO2. Better spatial characterization of both NO2 and NH3 concentrations, especially near intensive agriculture, would help to characterize the extent of the impacts across the U.S.

Sirkku Manninen, Faculty of Biological and Environmental Sciences, University of Helsinki; Jääskeläinen K., Faculty of Biological and Environmental Sciences, University of Helsinki; Niemi J., Helsinki Region Environmental Services Authority (HSY), Helsinki, Finland.

The structure of epiphytic macrolichen community in relation to modelled NO_2 concentration in a boreal city

Vandinther (2019) showed dry deposition of NO and NO_2 being a strong driver of lichen community structure on the acid bark of Jack pine (*Pinus banksiana*) in northern forests. The responses of microlichens to NH_3 and NO_x vary even within a given functional group. This is partly attributed to species-specific uptake rates of NH_4^+ cf. NO_3^- cf. organic N (Dahlman et al. 2004). Moreover, N-tolerant species can oxidize surplus NH_4^+ to NO_3^-, a non-toxic form of N, and thus do not accumulate excess N as NH_4^+ to same extent as acidophytes do (Gaio-Oliveira et al. 2004, 2005).

Epiphytic macrolichens were scored on *Pinus sylvestris* and/or *Quercus robur* trunks in Helsinki (60°10'N, 24°56'E) in summer 2016 using the Finnish standard (Suomen Standardisoimisliitto 1990), while the European Standard EN 16413:2014 was used in summer 2019. In 2016, the number of indicator species correlated negatively with modelled NO_2 concentration, SO_2 concentration, and concentrations of NO_2^-+NO_3^- -N, NH_4^+ -N and S of *Pinus* bark. The most responsive acidophytes to oxidized forms on N seemed to be e.g., *Hypogymnia physodes* and *Parmeliopsis ambigua* (Manninen 2018). Based on the 2019 data, *P. ambigua* on *Pinus* responded negatively to modelled NO_2 (range 8.0-11.9 µg m^{-3} yr^{-1}) as did *H. physodes* and the lichen diversity value of acidophytes (LDV_A) on *Quercus* (8.0-23.4 µg NO_2 m^{-3} yr^{-1}). In contrast, increases were found in the abundances and presence of nitrophytic species on *Quercus* with increasing NO_2 concentration. The results suggest a shift from the dominance of acidophytes to that of nitrophytes on at 10-15 µg NO_2 m^{-3} yr^{-1}. The results will also be discussed in terms of methodology (e.g., calculated indices).

Dahlman et al. 2004, Planta 219, 459-467
Gaio-Oliveira et al. 2004, Environmental Pollution 158, 2553-2560
Gaio-Oliveira et al. 2005, Planta 220, 794-803
Manninen S. 2018, Science of the Total Environment 613-614, 751-762
Suomen Standardisoimisliitto 1990. SFS Standard 5670. Air Quality. Bioindication. Mapping of Epiphytic Lichens (in Finnish).
Vandinther K. 2019. The influence of nitrogen deposition on community composition in Pinus banksiana forests across Northwestern Canada. M.Sc. thesis. Trent University, Peterborough, Ontario, Canada. 175 pp.

Duncan Mifsud, University of Kent

The Influence of NOx Emissions on the Nitrogen Isotopic Composition of Tree Rings and Foliage

Emissions from motor vehicle traffic over the past few decades have become a significant contributor to regional air pollution. Nitrogen oxides (NOx) are a major component of traffic emissions, and it is known that exposure to these species may be detrimental to public health. Recent studies have demonstrated that the stable isotope ratios of nitrogen in tree rings and foliage are influenced by the nature of their major nitrogen source, making them appropriate for semi-quantitative bio-monitoring studies. This proxy was applied to Aleppo pines (P. halepensis) growing at three distances from one of the busiest roads in Malta, a small country known to suffer from intense traffic pollution. No temporal variation in the nitrogen and organic carbon stable isotope ratios was detected in the sampled tree rings corresponding to the time period 1980-2018. However, statistically significant spatial trends were observed in both tree rings and foliage: sampled sites closer to the road exhibited more positive δ15N and more negative δ13C values compared to those at a rural background site. This is likely due to their increased take up of 15N enriched NOx and 13C depleted CO2 from traffic emissions. Top soils sampled at the three investigated sites also showed the δ15N trend. These results contribute to a growing body of evidence suggesting that tree ring and foliage isotope measurements are a useful indicator of regional air pollution and are also the first known application of dendrogeochemistry to atmospheric pollution monitoring in Malta.

Mike Perring, UKCEH

A brief exploration of current literature

Mike will present initial findings from a systematic search of the literature since the publication of NOx critical levels from the 1992 Egham workshop. He will highlight the themes that emerge from this brief review, including research on pollutant mixtures, effects on native as well as crop species, gradient studies, and the importance of NO in plant metabolism. He will provide a table of the main literature, and invite participants to consider whether they know of additional evidence that could be included in subsequent analyses (published or unpublished). He will also provide a framework for classification of the literature, which may help subsequent analyses and invite comment.

Helena Ribeiro, University of Porto

Effects of increasing nitrogen dioxide concentrations in pollen of different forest species

Pollen, the male gametophyte of seed plants, has a preponderant role in fruit production and consequently for the propagation of the species, as well as a food source for some pollinators. During emission and dispersion in the air, pollen is subjected to chemical and physical interactions with other atmospheric constituents, such as gas pollutants, which can cause stress in these biological structures and influence its mission. Therefore, pollen sensitivity and tolerance to air pollutants such as NO2 can be ultimately preponderant for crop production success. In this talk, it will be presented the influence of NO2 in key aspects related to pollen performance of 4 forest tree species, *Betula pendula*, *Corylus avellana*, *Acer negundo* and *Quercus robur*, through a comparative analysis under the same experimental conditions. We will discuss the effect on pollen fertility, protein content, oxidative stress, and wall composition after exposure in vitro to nitrogen dioxide at increasing concentration levels. Our results suggest changes in pollen viability, protein content and differential sensitivity related to ROS synthesis, NADPH oxidase activity as well as in wall composition. Our study points out that significant pollen functions could be compromised even at common air pollutant's concentrations.