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Final Report

Experimental and literature review of internationally proposed critical levels for ammonia to protect the vegetation

by:

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Final report

Experimental and literature review of internationally proposed critical levels for ammonia to protect the vegetation

Final Report comprising the state of the art and the results from an ammonia fumigation experiment and a field gradient study

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On behalf of the German Environment Agency

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Abstract: Experimental and literature review of internationally proposed critical levels for ammonia to protect the vegetation

The publication reports about an extensive review of the peer-reviewed literature and numerous ammonia monitoring studies that were released after the UN-ECE critical levels for ammonia had last been modified in the year 2009 (work package 1). The results were collated in a background document, reported and discussed with over 100 colleagues from Europe and overseas and the proceedings of a scientific workshop (work package 4) including 19 featured papers have meanwhile been made available in a separate publication (Franzaring & Kösler 2023). The proceedings will serve as a valuable backup reference for international policy makers, who are involved in the regulation and monitoring of potentially adverse effects of atmospheric ammonia. In order to generate new experimental evidence (work package 2) on the potential sensitivity of plants, an ammonia fumigation and a field gradient study had been performed with eight perennial and five annual nitrogen-sensitive species. The aim was to expose the plants to sub-chronic concentrations below 10 µg m^{-3}, that occur in regions with high livestock numbers and exceed the critical levels for the protection of nitrogen sensitive vegetation. In the first year, plants were fumigated in greenhouse chambers that were supplied with liquid instead of gaseous ammonia in order to generate five ammonia concentrations in the low range from 0 to 10 µg m^{-3}. Because it was too warm in the closed environment and the phenology was not developing like in the natural setting, the plants were moved to a natural ammonia gradient in the lee of a farm in the second year. Outdoors, plants showed a normal development and ammonia concentrations at the five selected stations were in the concentration range originally intended. The annuals, except one leguminous species, produced a higher shoot mass close to the farm giving clear evidence of a fertilisation effect of ammonia. The responses of the perennial species were not that clear and were modified by the effects of drought and cutting that had been employed in the first season. Only one species, namely Purple Moor Grass (*Molinia caerulea* (L.) Moench) showed a positive growth response to increasing ammonia levels. The species has also been reported to increase in presence and cover in areas subjected to strong eutrophication. We suggest that the nutrient ecology and life history traits of a plant species greatly affect its responsiveness to the uptake and metabolism of ammonia. Since our experiments only dealt with growth responses and nitrogen accumulation in single plants and the effects of nitrogen deposition will largely and on the long run affect the competition between fast and slow-growing plants, we suggest that future studies should be initiated in established vegetations. In work package 4, we thus recommend existing and new approaches for field fumigation and field gradient studies and for future research suggest that in depth studies should be performed in a peat and a fenland and in an acidic and a calcareous dry grassland to cover habitats that will probably show strongest adverse effects. Field experiments should be established at sites with existing infrastructure for global change research, while biomonitoring studies can be performed e.g. in citizen science projects with pre-cultivated plants and passive samplers in the lee of selected ammonia emitters.

Kurzbeschreibung: Experimentelle Überprüfung international vorgeschlagener Wirkschwellen (Critical Levels) von Ammoniak auf die Vegetation

Die Veröffentlichung berichtet über eine umfangreiche Sammlung der Peer-Review-Literatur und zahlreiche Ammoniak-Monitoring-Studien, die nach der letzten Änderung der UN-ECE-Grenzwerte für Ammoniak im Jahr 2009 veröffentlicht wurden (Arbeitspaket 1). Die Ergebnisse wurden in einem Hintergrunddokument vorgestellt und mit über 100 Kolleginnen und Kollegen aus Europa und Übersee diskutiert. Die Proceedings eines wissenschaftlichen Workshops (Arbeitspaket 4) inklusive der 19 Konferenzbeiträge wurden inzwischen in einer separaten Publikation (Franzaring & Kösler 2023) veröffentlicht. Dieses Dokument wird internationalen politischen Entscheidungsträgern, die an der Regulierung und Überwachung potenziell schädlicher Auswirkungen von atmosphärischem Ammoniak beteiligt sind, als wertvolle Referenz dienen. Um neue experimentelle Nachweise über die potentielle Empfindlichkeit von Pflanzen zu generieren (Arbeitspaket 2), wurde eine Ammoniakbegasung und eine Feldgradientenstudie mit acht mehrjährigen und fünf einjährigen stickstoffempfindlichen Arten durchgeführt. Ziel war es, die Pflanzen subchronischen Konzentrationen unter 10 µg m^{-3} auszusetzen, die in Regionen mit hohem Viehbestand auftreten und die kritischen Werte für den Schutz stickstoffempfindlicher Vegetation überschreiten. Im ersten Jahr wurden Pflanzen in Gewächshauskammern begast, die mit flüssigem statt gasförmigem Ammoniak versorgt wurden, um fünf Ammoniakkonzentrationen im niedrigen Bereich von 0 bis 10 µg m^{-3} zu erzeugen. Da es in der geschlossenen Umgebung zu warm war und sich die Phänologie nicht wie in der natürlichen Umgebung entwickelte, wurden die Pflanzen im zweiten Jahr in einen natürlichen Ammoniakgradienten im Lee eines Bauernhofes exponiert. Im Freien zeigten die Pflanzen eine normale Entwicklung und die Ammoniakkonzentrationen an den fünf ausgewählten Stationen lagen im ursprünglich vorgesehenen Konzentrationsbereich. Die Einjährigen, mit Ausnahme einer Leguminosenart, produzierten eine höhere Sproßmasse in der Nähe des Betriebs, was einen deutlichen Hinweis auf die Düngewirkung von Ammoniak gibt. Die Reaktionen der mehrjährigen Arten waren weniger deutlich und wurden durch die Auswirkungen von Trockenheit und einer Zwischenernte, die in der ersten Saison erfolgten, verändert. Nur eine Art, nämlich das Pfeifengras (*Molinia caerulea* (L.) Moench), zeigte eine positive Wachstumsreaktion auf steigende Ammoniakwerte. Es wurde schon des Öfteren berichtet, dass diese Art in Gebieten, die starker Eutrophierung ausgesetzt sind, an Präsenz und Deckung zunimmt. Wir bestätigen insgesamt, dass die Nährstoffnutzung und die ökologischen Eigenschaften einer Pflanzenart ihre Reaktion auf Ammoniak und dessen Stoffwechsel stark beeinflussen. Da sich unsere Experimente nur mit Wachstumsreaktionen und Stickstoffakkumulation einzelner Pflanzenarten sowie Pflanzenindividuen befassten und die Auswirkungen der Stickstoffdeposition langfristig die Konkurrenz zwischen schnell und langsam wachsenden Pflanzen beeinflussen werden dürfte, schlagen wir vor, zukünftige Studien in etablierten Vegetationen zu initiieren. In Arbeitspaket 4 empfehlen wir daher bestehende und neue Ansätze für Feldbegasungs- und Feldgradientenstudien und schlagen für die zukünftige Forschung vor, dass vertiefte Studien in einem Moor und einem Feuchtgrünland sowie in einem sauren und kalkhaltigen Trockenrasen durchgeführt werden sollten, um Lebensräume abzudecken, die wahrscheinlich die stärksten negativen Auswirkungen aufweisen werden. Feldexperimente sollten an Standorten mit vorhandener Infrastruktur für die Klimawandelforschung etabliert werden, während Biomonitoring-Studien z.B. in Citizen-Science-Projekten mit vorkultivierten Pflanzen und Passivsammlern im Lee ausgewählter Ammoniakemittenten durchgeführt werden können.

Table of content

List of figures

List of tables

List of abbreviations

AAQD	Ambient Air Quality Directive
BAFU	Bundesamt für Umwelt (CH, former BUWAL and BWG)
BD	Background Document
BImSchV	Bundesimmissionsschutzverordnung
CEH	Centre for Ecology and Hydrology (UK, several units)
CEN	Comité Européen de Normalisation
CFH	Core Facility Hohenheim
CLE	Critical Level for Air Pollutant Concentration, µg m^{-3}
CLO	Critical Load for Deposition, kg ha^{-1} yr^{-1}
CLRTAP	UNECE Convention on Long-Range Transboundary Air Pollution
CNP	Climate Normal Period
CRDS	Cavity Ring-Down sensors (CRDS)
CSTR	Continuously-Stirred Tank Reactors
CUT	Plants subjected to an intermediate harvest in 2020 to simulate mowing
DEFRA	Department for Environment, Food & Rural Affairs
DOAS	Differential Optical Absorption Spectroscopy
DOI	Digital Object Identifier
DWD	Deutscher Wetterdienst, German Weather Service
DT	Dry Treatment of Plants in the Fumigation Study, ca. 33% less than WW
ECN	Environmental Change Network
EMEP	European Monitoring and Evaluation Programme
EUNIS	European Nature Information System, URL: http://eunis.eea.europa.eu
FACE	Free Air Carbon Dioxide Enrichment
FKZ	Forschungskennzahl des UBA
HAR	Harvest of a Plant, Growth Determination on a Specific Date
HSD	Honestly significant difference between treatment means, Tukey Test
IED	Industrial Emissions Directive
IMH	Intermediate Harvest, representing a mowing half-way of the season (see CUT)
INI	International Nitrogen Initiative
INRA	Institut National de la Recherche Agronomique (France, several units)
IPC	The International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects e.g. on Forests
IPCC	Intergovernmental Panel on Climate Change
IPPC	Integrated Pollution Prevention and Control
IRMS	Isotope Ratio Mass Spectrometry
LAI	Bund/Länder-Arbeitsgemeinschaft für Immissionsschutz
LANA	Bund/Länder-Arbeitsgemeinschaft Naturschutz
LANUV	Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen
LfU	Bayerisches Landesamt für Umwelt

LTER	Long-Term Ecological Research Network
LRT	Lebensraumtyp (Habitat Type according to Natura2000)
LRTAP	UNECE Convention on Long-range Transboundary Air Pollution
LTZ	Landwirtschaftliches Technologiezentrum Baden-Württemberg
LUBW	Landesanstalt für Umwelt Baden-Württemberg
MAK	Maximale Arbeitsplatz Konzentration (permissible workplace concentration)
NA	Data not available
NEC	National Emission Ceilings
NERC	National Emissions Reduction Commitments, Directive (EU) 2016/2284
NS	Difference of treatments non-significant, based on a statistical test
NUE	Nitrogen use efficiency
OTC	Open Top Chamber
PHT	Phytotechnikum Hohenheim
PINETI	Pollutant Input and Ecosystem Impact, UBA Project on N-Deposition
PM	Particulate Matter, e.g. PM_{10}, $PM_{2.5}$
PRTR	Pollutant Release and Transfer Register of the EU
ReFoPlan	Ressortforschungsplan
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (NL, Bilthoven)
RR	Response ratio of plants subjected to the highest vs. the lowest concentration
RSR	Root:Shoot ratio
SEN	Senescent Leaf Mass (collected several times throughout the experiment)
SHG	Serviceeinheit Hohenheim Gewächshäuser
SMB	Simple Mass Balance Model for Nitrogen
SPAD	Soil Plant Analysis Development (SPAD) chlorophyll meter
TA Luft	Technische Anleitung zur Reinhaltung der Luft
TOT	Total Shoot Mass of a Plant
UBA	Umweltbundesamt (German Environment Agency)
ULI	Unterer Lindenhof (Farm of the University of Hohenheim)
UNCUT	Plants not subjected to an intermediate harvest in 2020
UNECE	United Nations Economic Commission for Europe
UPB	Umweltprobenbank des Bundes
VDI	Verein Deutscher Ingenieure (German Board of Engineers)
VMM	Vlaamse Milieumaatschappij (B)
VPD	Vapour pressure deficit
WP	Work Package
WUE	Water use efficiency of plants; shoot biomass divided by water supply (g L^{-1})
WW	Well-Watered Plants of the Fumigation Experiment 2020
WWTP	Waste Water Treatment Plant

Summary

As budgeted by the departmental research plan (ReFoPlan) of the Federal Environment Agency (UBA), a project with the title *"Experimental and literature review of internationally proposed critical levels for ammonia to protect the vegetation"* was performed by the University of Hohenheim between 2019 and 2023. The project consisted of four work packages, a literature survey (WP1), an ammonia fumigation experiment under controlled conditions (WP2), a feasibility study for field and transect experiments (WP3) and the preparation and summary of an international expert workshop (WP4), which was held in Dessau on 28 and 29 March 2022.

While WPs 1 to 3 are described in detail in the present report, the results of WP4 are available in the Conference Proceedings that have been published recently in the UBA-series TEXTE 31/2023 (Franzaring & Kösler, 2023). The latter document is a stand-alone project and workshop output and will serve as a valuable backup reference for scientists and policy makers, who are involved in the regulation, study and monitoring of potentially adverse effects of atmospheric ammonia.

The background and reason for the initiation of the project was the growing environmental and economic importance of the air pollutant ammonia and the question whether the critical levels (CLE) that had been suggested by the United Nations Economic Commission for Europe (UNECE) in 2009 needed to be updated. To date, the gas mainly stems from agricultural sources and together with the oxidised forms of nitrogen it is significantly contributing to the widespread eutrophication and acidification of ecosystems. Ammonia emissions will have to be reduced significantly in Germany and elsewhere in the EU according to the national emission ceilings laid down by the updated NEC Directive 2016/2284/EU and the 2020/2030 national reduction commitments (NERC). Currently, it is unclear how far these emissions will go down due to the enforcement of the existing regulations.

The basis for the current CLE was laid by the work of Cape et al. (2009a), which provided evidence to reduce the long-term critical level of ammonia from a previous value of 8 down to 3 µg m^{-3} and to 1 µg m^{-3} for N-sensitive vascular plant species and the lichen and bryophyte (moss) vegetation (i.e. lower species), respectively. CLE had almost exclusively been based on research from Scotland, e.g. the long-term Whim Bog fumigation experiment. In the year 2009, only few studies from other, more densely populated and agricultural regions were available although the background concentrations and the environmental impacts of the gas are much higher. Long-term effects of ammonia deposition on N-sensitive ecosystems are probably more pronounced in other regions than the UK.

We can assume that background levels of ammonia are exceeding the suggested critical levels in the traditional European intensive livestock belts (Northern France, The Po Valley, Flanders, the Netherlands and Northern Germany) and in the European metropolitan areas. Constantly monitoring ammonia concentrations in the lower microgram range is cost intensive and technically challenging, but will probably have to be a backbone of an efficient air quality control of the gas in the future. However, ammonia is not yet covered by the European air quality directives (AQDs) equally as other main air pollutants.

The first part of the present report (**WP1**, chapter 2) gives an overview of the scientific knowledge that has been generated during the past decade. We retrieved 103 peer-reviewed references (until 2020) that cited Cape et al. (2009a) and collected relevant grey literature which we were hinted to through a survey we made among 57 experts from 14 European countries. We also asked these experts about how the ecological relevance of ammonia is being evaluated, assessed and measured in their home countries. The references were retrieved from Scopus® and collated in a database (Citavi).

Most of the material published in peer reviewed journals stems from the UK, Canada and China and deals with general effects of N-deposition on biodiversity and modelling issues, too often without a clear differentiation between oxidized and reduced forms of nitrogen and how much the gas ammonia actually contributes to potential adverse effects. Astonishingly, only few publications stem from Germany, France, Denmark and the Netherlands, i.e. countries where ammonia emissions are high compared to e.g. Northern Europe. In contrast, many citations of Cape et al. (2009a) came from the Mediterranean region, mainly by scientists, who investigated the lichen diversity along ammonia gradients.

Plant fumigation studies with controlled concentrations of the air pollutant at question are lacking to derive reliable dose-response curves, but are the basis for the establishment of environmental standards including CLE. Using natural and artificial ammonia gradients like in the lee of livestock stables and in the Whim Bog experiment are meaningful to address adverse effects in the field, but atmospheric, climatic and edaphic conditions vary along theses gradients and may confound with the effects of ammonia. Furthermore, true replications for the construction of reliable dose response functions are unavailable in gradient studies.

In general, the material published recently does not give much new information on the validity of critical levels. While some studies confirmed the high susceptibility of lichens to ammonia and other nitrogen containing air pollutants, only few studies investigated the responses of vascular plant species in the lee of livestock farms. It also remains unclear if and how physiological responses translate into ecological effects. It also needs to be discussed in general what will be the ecological relevance if rare oligotrophic lichen species were replacing the widespread and opportunistic eutrophic flora following the reduction of ammonia emissions.

Only one reference was published after 2009, reporting on a study in which plants were experimentally exposed to ammonia in a controlled manner. Unfortunately, most publications do not give details on how the experiment was performed. Critical points refer to the use of undiluted pure ammonia and the technical problems related to the continuous online-monitoring of concentrations in the lower range.

In contrast to the widely observed lack of dose-response studies large efforts have been made in recent years in various countries to monitor environmental ammonia. We found several papers and recent reports on the results from routine monitoring programs using passive samplers. Prominent examples are the long-term studies in the Netherlands, the UK, Switzerland and Belgium, where ammonia networks have been established in a number of Natura2000 areas. National and regional networks have also been established in the US and Canada, but also Germany has made some progress. Other large European ammonia emitters, however, lag severely behind and have not established routine-like monitoring systems.

A European Project and a ring trial at Whim Bog confirmed the suitability of different passive sampler types for the determination of slightly elevated levels of ammonia, but in the lower microgram range large measurement uncertainties prevent the exact monitoring. Other methods with a better temporal resolution than passive samplers are currently being developed to accurately determine low concentrations of ammonia and the potential exceedance of critical levels. Differential optical absorption spectroscopy (DOAS) and Cavity Ring-Down sensor (CRDS) systems are promising new technologies and are currently being used at a few locations for the time-resolved, continuous online monitoring of ammonia in the Netherlands, the UK, Germany and Switzerland.

Conflicting with the results from national emission inventories and nitrogen deposition models, monitoring of ammonia concentrations in the above-mentioned countries has shown that levels of the gas have not exhibited downward trends during the last decade. In most of the countries, ammonia concentrations are increasing and two reasons have been made responsible for the general rise. Firstly, the strong reduction of SO_2 emissions over the past three decades has led to less formation of secondary particles $(NH_4)_2SO_4$ and has increased the lifetime and long-range transport of ammonia. Secondly, the elevated temperatures due to climate change have been made responsible for higher evaporation rates of ammonia from plant surfaces, soils and waters. It is unclear to date, whether other emission sources namely the generation of bioenergy, compost and waste treatment facilities and the growing use of diesel exhaust fluids (DEF) contribute to the rising levels of atmospheric ammonia. However, we mention several references which show that ammonia emissions from (diesel) vehicles have played a greater role in recent years. We also hint here to the potential emissions from ammonia storage containers, which will be used in the nearer future to increase the supply of hydrogen.

In order to ascertain whether and how the suggested UN-ECE critical levels for ammonia are being applied in Europe, we undertook a survey among 57 experts from 14 countries. There were ten replies from six countries including information on how ammonia is dealt with at the local and regional level was very scarce. Ammonia legislation and control is only in force in the Netherlands, Denmark, Germany and the UK, while other countries have obviously not realised the environmental problems related to ammonia. No one of the respondents knew of further current scientific work experimentally studying the effects of ammonia in fumigation chambers. However, the contact at CEH Edinburgh provided some new publications from the world-wide unique Whim Bog Experiment and we obtained some recent information on projects in the UK and overseas.

In **WP2**, we aimed to generate new information on the response of nitrogen sensitive plant taxa to ammonia using targeted experiments. The approach should make use of a controlled fumigation system producing five ammonia treatments in the range from 0 to 10 µg m^{-3}, i.e. low concentrations representative of environmental levels at which chronic responses would begin to evolve in sensitive plant communities. Experiments required the use of N-sensitive endangered plant species and two experimental seasons. The classical ecotoxicological approach should be able to derive dose-response relationships between ammonia concentrations (exposure) and relevant endpoints, i.e. biological effects like growth responses.

Extensive considerations were made about the source of ammonia and exposure method to be used in the fumigation experiment. We wanted to refrain from using technical (gaseous) ammonia supplied directly from pressure flasks because the accidental release from flasks used in closed environments poses a severe health risk to the people working in the greenhouse. At the same time, unrecognised peak concentrations could harm the plants and the aluminium structures of the greenhouse compartments. We therefore decided to work with diluted (harmless) chemical solutions, aqueous ammonia (NH_4OH), which allowed the gentle and complete evaporation of ammonia over time. Knowing the exact amount of ammonia (in the liquid) and the volume and air ventilation rates of the chambers enabled us to calculate and set the mean concentrations. In total, five levels were targeted at in the range from 0 to 10 µg m^{-3}.

In the first year, the season 2020, experiments were performed in five compartments of the Phytotechnikum Hohenheim (PHT) greenhouse facilities. For the monitoring of realized ammonia concentrations, we chose Radiello® passive samplers because they are widely used and have a radial geometry of the absorbing material that allows for a high collection rate of the gas. Sampling intervals could thereby be shortened to a week instead of two or four weeks in other passive samplers.

A gentle and time-controlled evaporation of diluted solutions was performed to tune in low concentrations of ammonia in four greenhouse compartments. The fifth greenhouse unit was used as a control, where no ammonia was applied to the plants. The treatments were rotated bi-weekly between the four fumigated compartments to avoid placement effects. For the supply of liquid ammonia to the four compartments, we used a multi-channel peristaltic pump and long Teflon® tubes. Drops of liquid ammonia were vaporised in each of the chambers using fans.

For the plant study, we aimed to expose the plants to five levels of ammonia and to subject half of the plants to a drought treatment to experimentally address interactions between eutrophication and climatic change. The plant species selected were supplied as seeds from different suppliers and were sown in the autumn 2019. *Koeleria glauca* (Schrad.) DC. (Blue Hair Grass), *Antennaria dioica* (L.) Gaertn. (Pussytoes), *Dianthus deltoides L*. (Maiden Pink), *Carex arenaria* L. (Sand Sedge) and *Arnica montana* L. (Wolf´s Bane) were grown from commercially available germplasm, while from latter species we were able to obtain seeds from *Nardus* grasslands. Since for *Arnica*, a characteristic species of acidic montane grasslands, we could cultivate plants from two seed origins, we could investigate whether those would show a different response to ammonia. Besides these five seed-grown species, we propagated ramets of *Molinia caerulea* (L.) Moench (Purple Moor Grass) and purchased plants of *Pulsatilla vulgaris* Mill. (Pasqueflower). Since we used ten replicates per ammonia treatment and drought sub-treatment, we needed plant individuals. Plants were grown in 1L plastic pots and the substrate used was made up from a standard earth and soil material obtained from natural sites where the selected plant species grow. A fertilisation with ^{15}N labelled NH_4NO_3 was made at the start of the experiment, but stable isotopes were only addressed in *Arnica* and *Molinia,* the latter species known to respond positively to eutrophication.

The greenhouse fumigation experiments were performed during the vegetation period of the year 2020, therefore started in May 2020 and lasted until October 2020. The pump and the tubes for the ammonia exposure worked without failure during the five months of the fumigation study and ammonia concentrations and average temperatures were monitored in each of the chambers throughout the study. While ammonia data had a weekly resolution due to the use of passive samplers, temperature and humidity were logged on an 1h basis. Ammonia concentrations varied throughout the experiment and averaged to 5.2 in the control to 17.6 µg m^{-3} in the highest treatment. The plants were thus exposed to somewhat higher concentrations than those originally envisaged. At the same time, the temperatures recorded in the greenhouse compartments were higher by 6.7°C as compared to outdoors and peaks of over 40°C afforded a higher supply of water. Nevertheless, we were able to realize a drought treatment with on average 33% less water to half of the plants. Despite the insect infestations on Arnica, all species developed healthy plants and only a few individuals died during the greenhouse experiment. We are thus sure that the results of our experiment were reliable in general, but it must be noted that the phenology appeared to be changed since most of the plants did not enter the stage of flowering due to a lack of vernalization.

Shoot biomass was assessed at an intermediate and a final harvest. All of the species showed a significant drought effect on the shoot biomass in both harvests, while the effects of ammonia were significant in only a few species at the intermediate and the final harvest of the first year. Analyses of variance and *post-hoc* multiple comparisons (Tukey tests) confirmed many significant differences between the treatments, but we did not observe linear responses from the lowest to the highest concentration. This may have been due to the fact that the range of realized concentrations did not feature large differences between the intermediate levels, so it was not possible to derive dose-response curves. We therefore calculated the response ratio (RR), i.e. the biomass produced in the highest (17.6 µg m^{-3}) as related to the lowest (5.2 µg m^{-3}) ammonia concentrations and addressed growth responses of larger than 20%. Interestingly, growth stimulations of over 20% were absent in the first year, while growth reductions due to the elevated ammonia were seen in the dry treatments of *Antennaria dioica*, *Koeleria glauca*, *Carex arenaria* and *Dianthus deltoides* in both cut and uncut plants. However, in the productive origin of

Arnica montana, the well-watered cut plants showed to produce less biomass in the elevated ammonia treatment.

A second experimental season followed the greenhouse study to investigate the effects of ammonia on the performance of perennial plants that were previously subjected to combinations of drought and cutting (mowing). In order to keep the plants nutrient limited, we did not fertilise the pots in spring 2021. Since in the first year, plants had been growing at temperatures almost 7°C higher than outdoors, we moved the plants into the field in the second year. Five stations were selected in the lee of the "Unterer Lindenhof", a farm belonging to the University of Hohenheim, representing a natural climate and a typical ammonia gradient. The seasonal ammonia concentrations in the field ranged from 1.8 downwind to 9.4 µg m^{-3} close to the farm and well represented the concentration range we originally were aiming at. However, the three proposed intermediate ammonia levels only ranged from 2 to 4.7 µg m^{-3} and like in the previous year, did not create step-wise linear responses in the plants. Nevertheless, the climate was more or less comparable to the long-term situation and manually watering at drier times ensured a normal development of the plants. In addition to the perennial plant species, we also exposed five pre-cultivated annual plant species, namely *Iberis amara*, *Trifolium arvense*, *Bromus hordeaceus*, *Erodium cicutarium* and the rare red-listed *Legousia speculum-veneris*. Plant selection was based on functional properties and taxonomy and their restriction to nitrogen-poor habitats. In the second year of the experiments, neither the perennials nor the annuals were subjected to a drought treatment and harvests were performed only once at the end of the season. However, plant exposures and harvests of the 13 plant species differed according to their phenology. In the end of the season, shoot biomass could be related to the different ammonia levels that were registered at the five stations for the duration of the exposure of plants.

In order to understand the growth responses of perennial species to ammonia, it is important to consider their different ecology and life history traits. While the three species *Koeleria glauca*, *Dianthus deltoides* and *Pulsatilla vulgaris* produced >30% less biomass in the second season, *Antennaria diocia*, *Carex arenaria* and *Molinia caerulea* were able to produce >40% more shoot mass despite no fertilisers were added to the pots in the second season. The innate, i.e. genetically-fixed, capacity to acquire resources for growth at limited conditions will have a strong influence on the long-term performance of plant species. The strongest increase in biomass although only the third most productive in absolute terms, was observed in *Molinia caerulea*, a grass species that has a wide ecological amplitude and which has increased much in heathlands in regions with eutrophication problems. Ammonia significantly increased the shoot in each of the sub-treatments, irrespectively of whether the plants had been grown under dry conditions or had been subjected to an intermediate cut in the previous season. We conclude that the potential stress *Molinia* receives by mowing and drought, i.e. the removal or missed build-up of resources, will not reduce the uptake and use of ammonia for the production of new biomass. In fact, plants, which were cut in the first season, produced more shoot biomass when at the same time they had been subjected to dry conditions. The results suggest that the root stock of the tussock-forming grass must be regarded as an important modifier to growth responses to drought, biomass removal and ammonia. We assume that the nutrient ecology of the sweet grass and the allocation of high proportions of biomass to its rootstock enable *Molinia caerulea* to double its biomass in the second year.

A similar increase in shoot mass from the first to the second year was observed in the sedge *Carex arenaria*. In contrast to *M. caerulea*, the species was not able to increase growth with the supply of ammonia. In both years, the uncut plants from the dry treatment showed a decreased shoot mass under elevated ammonia. We are not sure about the underlying mechanism, but it may be suggested that the stolon-forming species needs nutrient-poor conditions to form new ramets. A likewise behaviour may be the reason for the reduced shoot mass of *Koeleria glauca*, *Dianthus deltoides* and *Pulsatilla vulgaris*. Since the shoot mass of the three species was much lower in the second than in the first year, we can assume that these species will not be long-lived under a low nutrient supply. But it may be possible that other nutrients like P, K and Ca will be more limiting than the supply of N. One must also hint to the fact that some plant species prefer NO_3-N over NH_4-N.

Contrasting results were found in the two composite species *Antennaria dioica* (Pussytoes) and *Arnica montana* (Wolf´s Bane). While in Pussytoes, the exposure to elevated ammonia decreased the shoot mass of uncut plants, shoot mass tended to increase in the commercial line of Arnica. However, many individuals of the species died during the experiments, so we are not sure about the representativeness of the results.

While the results of growth analyses were diverse in the perennials, three out of five annuals showed an increase in shoot mass when grown close to the stables. Obviously, they were able to make use from ammonia, while the slow-growing *Legousia speculum-veneris* showed no clear responses and *Trifolium arvense* a 32% reduction in shoot mass. We therefore suggest that the growth of short-lived species, often the ruderals, may increase growth due to ammonia fertilisation. The negative response of *Trifolium arvense* (Rabbitfoot Clover) hints to the fact that N-fixing species might respond differently to the supply of ammonia. We therefore suggest to study further leguminous species in the future.

In order to be able to trace the uptake and remobilisation of nitrogen, all plants were lightly fertilised with a nutrient solution enriched in ^{15}N before the start of the fumigation experiment. The uptake of nitrogen from ammonia – volatilised from liquid ammonia, it contains higher proportions of the lighter isotope – was hypothesised to lead to a ^{15}N-dilution over time. We performed analyses of ^{15}N and nitrogen contents of the different shoot fractions (senescent leaves and stems) of the two origins of *Arnica montana* and *Molinia caerulea*. Since roots are the biggest organs of grasses, we also analysed N concentrations and isotopes in latter species, the Purple Moor Grass.

The dilution of ^{15}N over time, i.e. the lowering of δ, was well visible in senescent leaf fractions of both Arnica origins that were collected during the first year. The signatures went down from the set value of 100 in leaves that senesced first to 30 in the fraction that senesced only in the winter. Initially, the ^{15}N dilution was less pronounced in the plants that were grown under drier conditions, but this appeared to be reverted later in the season. The results confirmed that plants, whose growth conditions are worsened by stress will first use the existing resources and only take up significant quantities of atmospheric ammonia later. While this effect could be seen in the senescent leaves that were shed in the first season, in the second year the isotopic signatures did not differ any more between plants from the dry and the well-watered treatment. Nevertheless, isotopic signatures differed between the two Arnica populations, with the more productive commercial origin showing a stronger ^{15}N-dilution than the slower growing plants from the wild population. When comparing the nitrogen concentrations of plant fractions and the total nitrogen that was present in the shoot mass grown under different exposure to ammonia, we did not identify any effects of ammonia. However, the plants from the wild population had only half of the nitrogen on a pot basis than those from the fast-growing commercial seed origin of Wolf Bane.

Much of the acquisition of NH_4^+ from deposited gaseous ammonia happens via the plant´s surface and it is likely that upright leaves and stalks of grasses will be able to scavenge more ammonium from the atmosphere than flat-leaved stocky herbs. Indeed, two of the grasses we used in our study, showed more vivid growth close to the farm as a consequence of the higher ammonia concentrations. Although the shoot N-concentrations in *Molinia caerulea* stayed below 0.6%

irrespectively of the distance to the farm, the nitrogen mass that was present on a pot basis decreased along the gradient with increasing distance. It must be noted that plants that had been cut in the previous season, had a lower quantity of nitrogen per pot than those which were not subjected to an intermediate harvest. Although we saw a stronger ^{15}N dilution in the shoots of both cut and uncut plants that grew close to the stable, we did not observe a linear relationship to the mean ammonia concentrations. Interestingly, ^{15}N dilution was less pronounced in the rootstocks confirming that much of the nitrogen fixed in the root mass still stemmed from the fertilisation that was applied at the start of the experiment a year before.

Our experiments were based on a single-plant-in-pot-study and are not necessarily representative for plants growing in co-existence with other individuals and plant species. Although it is important to know about taxonomic differences in the sensitivity of different plant species, it will be necessary to address ammonia-driven changes in plant communities at natural sites. In **WP3**, a feasibility study should be performed for the study of ammonia in established vegetation. The most interesting habitats and ecosystems will be those that have developed in nutrient poor conditions, e.g. heaths, bogs and grasslands on shallow acidic substrates. Since ammonia is supposed to favour the fast-growing productive species, small herbs will be outcompeted by the dominant often the grassy species. Apart from field-based fumigation experiments, we also addressed the study of plants in ammonia gradients along the lee of emitters and the feasibility to use bioindicators, e.g. in citizen science experiments.

While open-top chambers (OTC) have been used as a standard technology to study the effects of air pollution (SO_2, O_3 and NH_3) in established vegetations, only one chamber less field manipulation experiment is available worldwide, which provides a quantified ammonia concentration (deposition) gradient to an ombrotrophic bog under natural climates. Experiments had been launched in the Scottish Whim Bog in 2002 and the site is now part of the Horizon 2020 EU programme eLTER - European Long-term Ecosystem Research. Comparable fumigation systems have recently been built up by the operator (CEH Edinburgh) in forests in Scotland and Sri Lanka. Despite the shortcomings of the release of pure ammonia into an established vegetation and the very high concentrations and NHx deposition close to the outlet, the system is probably the best available technology currently available.

Instead of releasing undiluted ammonia via active fumigation into the vegetation, field exposure systems could be developed that rely on the passive and gentle volatilisation of diluted liquid ammonia from porous tubes. Time-controlled pumps could be used to resupply the liquid and create concentration gradients, but a roof and a trough must protect the tubes from rain and from leaking/leaching liquid ammonia into the plots.

Under the provision of a substantial funding of the equipment, running costs and technical and scientific staff, it will be possible to set up a similar or improved system like that one developed by CEH. It would be desirable to have two systems in a mire and a fen in the North and the South of Germany, respectively, and two more facilities in an acidic and a calcareous grassland. Forests will have to be excluded from ammonia fumigation experiments, but we advise to have four German sites with ammonia experiments in low growing vegetation.

Potential field sites for the experimental ammonia exposure should be considered at locations where long-term ecological research has and still is being performed. Ideally, there should be a research station and supply of electricity close by. Various networks have been identified that deal with global change research in vegetations, e.g. the study of bi-directional fluxes of climate relevant gases and the restoration of moorlands. The atmospheric component ammonia and the effects of eutrophication could be integrated into some of these running programmes.

We finally also addressed gradient studies in the lee of large livestock facilities. To limit potentially adverse effects on nature, large stables with many animals and presumably high ammonia emissions are licensed only at locations away from nitrogen sensitive targets. It will thus not be possible in such places to address effects of ammonia on the established natural vegetation. We therefore suggest to apply a similar approach as has been used in WP2. Pre-grown N-sensitive plant species can be exposed as bioindicators along the ammonia gradient in the lee of large stables. Besides annual plant species that showed positive growth responses in our experiment, a promising perennial would be the grass *Molinia caerulea*, which also showed ammonia driven changes in growth. Since we worked in the low concentration range between 2 and 10 µg m^{-3}, in which adverse effects on the plants were not observed, it will also be interesting to address higher sub-acute concentrations of > 30 µg m^{-3} typical in the lee of larger stables. It may well be that such concentrations may cause physical harm to vascular plants. Apart from using single-plant-in pot studies, we also advise using species mixtures, which might give deeper insights into the ammonia-driven changes in competitive abilities and long-term changes in the vegetation. Using a grass-clover mixture, e.g. *Molinia caerulea* growing with a *Trifolium* species, as a bioindicator could be a rewarding combination of a species showing a positive response with a species that does not make use from ammonia. After testing various plant combinations in the lee of stables, the best approach can be transferred to other study sites. We advise to develop such bioindication approaches that can be used e.g. in national citizen science projects and international co-operative projects like ICP vegetation.

Zusammenfassung

Das vorliegende Projekt "Experimente und Überprüfung international vorgeschlagener kritischer Werte für Ammoniak zum Schutz der Vegetation" wurde im Rahmen des Ressortforschungsplans (ReFoPlan) des Umweltbundesamtes (UBA) zwischen 2019 und 2023 von der Universität Hohenheim bearbeitet. Das Projekt bestand aus vier Arbeitspaketen, einer Literaturrecherche (AP1), einem Ammoniakbegasungsexperiment unter kontrollierten Bedingungen (AP2), einer Machbarkeitsstudie für Feld- und Transektexperimente (AP3) sowie der Vorbereitung, Durchführung und Zusammenfassung eines internationalen Expertenworkshops (AP4), der am 28. und 29. März 2022 in Dessau stattfand.

Während die APs 1 bis 3 im vorliegenden Bericht ausführlich beschrieben werden, sind die Ergebnisse von WP4 in einem kürzlich in der Reihe UBA-Texte 31/2023 (Franzaring & Kösler, 2023) veröffentlichten Tagungsband verfügbar. Das Dokument ist ein eigenständiges Projekt- und Workshop-Ergebnis und wird als wertvolles Hintergrunddokument für Wissenschaftler und politische Entscheidungsträger dienen, die an der Regulierung, Untersuchung und Überwachung potenziell schädlicher Auswirkungen von atmosphärischem Ammoniak beteiligt sind.

Hintergrund und Anlass für die Initiierung des Projekts war die wachsende ökologische und ökonomische Bedeutung des Luftschadstoffs Ammoniak und die Frage, ob die von der Wirtschaftskommission der Vereinten Nationen für Europa (UNECE) im Jahr 2009 vorgeschlagenen kritischen Werte (critical levels, CLE) aktualisiert werden müssen. Das Gas stammt zu 95 % vor allem aus der Landwirtschaft und trägt zusammen mit den oxidierten Stickstoffformen maßgeblich zur weit verbreiteten Eutrophierung und Versauerung bei. Die Ammoniakemissionen müssen in Deutschland und anderswo in der EU gemäß den nationalen Emissionsminderungsverpflichtungen der aktualisierten NEC-Richtlinie 2016/2284/EU bis zum Jahr 2030 deutlich herabgesetzt werden. Zwar gibt es mit dem Nationalen Luftreinhalteprogramm einen Maßnahmenkatalog und einen Fahrplan zur Erreichung der Minderungsverpflichtungen, dennoch ist unklar, ob die Emissionen tatsächlich im erforderlichen Maße sinken werden.

Grundlagen für die CLE wurden unter anderem durch die Arbeit von Cape et al. (2009a) gelegt, die nahe legen, dass die Ammoniakkonzentrationen zum Schutz von empfindlichen höhere Gefäßpflanzenarten und der Flechten- und Moosvegetation (d.h. niedere Pflanzenarten) Werte von 3 µg m^{-3} und 1 µg m^{-3} nicht mehr überschreiten dürfen. Die kritischen Werte basieren zum Großteil auf Forschungsarbeiten aus Schottland, z.B. dem Langzeitexperiment Whim Bog, in dem ein Moor mit Ammoniak begast wird. Im Jahr 2009 lagen nur wenige Studien aus den dichter besiedelten und stärker landwirtschaftlich geprägten Regionen vor, obwohl die Hintergrundkonzentrationen und die Umweltauswirkungen des Gases zumeist deutlich höher als in Schottland sind.

Wir können davon ausgehen, dass die Hintergrundwerte von Ammoniak die empfohlenen kritischen Werte in den traditionellen europäischen Viehregionen (Nordfrankreich, Poebene, Flandern, Niederlande und Norddeutschland) und in den europäischen Ballungsräumen überall überschreiten. Die ständige Überwachung von Ammoniakkonzentrationen im unteren Mikrogrammbereich ist kostenintensiv und technisch anspruchsvoll, muss aber gegebenenfalls in Zukunft das Rückgrat einer effizienten Luftreinhaltung des Gases sein. Ammoniak fällt jedoch bislang nicht unter die europäischen Luftqualitätsrichtlinien (AQDs).

Der erste Teil des vorliegenden Berichts (WP1, Kapitel 2) gibt einen Überblick über die wissenschaftlichen Erkenntnisse, die in den letzten zehn Jahren generiert wurden. Wir haben 103 Peer-Review-Artikel (bis 2020) finden können, die die Studie von Cape et al. (2009a) zitierten, und haben relevante graue Literatur gesammelt, auf die wir durch eine Umfrage unter 57

Experten aus 14 europäischen Ländern hingewiesen wurden. Wir haben diese Experten auch befragt, wie die ökologische Relevanz von Ammoniak in ihren Heimatländern bewertet und überwacht wird. Die Referenzen wurden aus Scopus® abgerufen und in einer Datenbank (Citavi bzw. EndNote™) zusammengeführt.

Das meiste Material, das in Peer-Review-Zeitschriften veröffentlicht wurde, stammt aus Großbritannien, Kanada und China und befasst sich mit allgemeinen Auswirkungen der N-Deposition auf die Biodiversität und Modellierungsfragen, zu oft ohne eine klare Unterscheidung zwischen oxidierten und reduzierten Formen von Stickstoff vorzunehmen und wie stark das Gas Ammoniak tatsächlich zu möglichen nachteiligen Auswirkungen beiträgt.

Erstaunlicherweise stammen nur wenige Publikationen aus Deutschland, Frankreich, Dänemark und den Niederlanden, also Ländern, in denen die Ammoniakemissionen im Vergleich zu z.B. Nordeuropa hoch sind. Im Gegensatz dazu kamen viele Zitate von Cape et al. (2009a) aus dem Mittelmeerraum, hauptsächlich von Wissenschaftlern, die die Flechtendiversität entlang von Ammoniakgradienten untersuchten. Begasungsstudien von Pflanzen mit unterschiedlichen Konzentrationen des betreffenden Luftschadstoffs fehlen größtenteils, so dass Dosis-Wirkungs-Kurven nicht ermittelt werden konnten. Letztere bilden aber die Grundlage für die Festlegung von Umweltstandards einschließlich der Ableitung von kritischen Werten.

Die Verwendung natürlicher und künstlicher Ammoniakgradienten wie im Lee von Viehställen und im Whim Bog-Experiment ist generell sinnvoll, um nachteilige Auswirkungen auf die natürliche Vegetation zu erkennen, aber atmosphärische, klimatische und edaphische Bedingungen variieren entlang von Belastungsgradienten und modifizieren die eigentlichen Auswirkungen von Ammoniak. Darüber hinaus sind echte Wiederholungen für die Ableitung von statistisch basierten Dosis-Wirkungs-Funktionen in solchen Gradientenstudien meist unzureichend verfügbar.

Im Allgemeinen geben neuere Publikationen nicht viele neue Informationen über die Gültigkeit kritischer Werte. Während einige Studien die hohe Anfälligkeit von Flechten gegenüber Ammoniak und anderen stickstoffhaltigen Luftschadstoffen bestätigten, untersuchten nur wenige Studien die Reaktionen höherer Pflanzenarten im Windschatten von Viehzuchtbetrieben. Es bleibt auch unklar, ob und wie physiologische Reaktionen in ökologische Effekte umgesetzt werden. Es muss auch allgemein diskutiert werden, welche ökologische Relevanz es hätte, wenn heute seltene oligotrophe Flechtenarten die mittlerweile weit verbreitete und opportunistische eutrophe Flora nach der Verringerung der Ammoniakemissionen wieder ersetzen würden. Nach 2009 wurde nur eine Referenz veröffentlicht, die über eine Studie berichtete, in der Pflanzen experimentell kontrolliert Ammoniak ausgesetzt wurden. Leider geben die meisten Publikationen keine Details darüber, wie die Experimente durchgeführt wurden. Die Verwendung von unverdünntem reinem Ammoniak und die technischen Probleme im Zusammenhang mit der kontinuierlichen Online-Überwachung von Konzentrationen im unteren Bereich sind kritisch anzumerken.

Im Unterschied zu insgesamt recht wenigen kontrollierten Dosis-Wirkungsexperimenten, wurden in den letzten Jahren in verschiedenen Ländern große Anstrengungen unternommen, um Ammoniak in der Umwelt zu messen. Wir fanden insgesamt viele Artikel und aktuelle Berichte über die Ergebnisse von Routineüberwachungsprogrammen mit Passivsammlern. Prominente Beispiele sind Langzeitstudien in den Niederlanden, im Vereinigten Königreich, in der Schweiz und in Belgien, wo in einer Reihe von Natura-2000-Gebieten Ammoniakmessnetze eingerichtet wurden. Auch in den USA und Kanada sind nationale und regionale Netzwerke entstanden, aber auch Deutschland hat einige Fortschritte gemacht. Andere große europäische

Ammoniakemittenten hinken jedoch stark hinterher und haben keine routinemäßigen Überwachungssysteme eingerichtet.

Ein europäisches Projekt und ein Ringversuch im schottischen Whim Bog bestätigten die Eignung verschiedener Passivsammlertypen zur Bestimmung leicht erhöhter Ammoniakwerte, aber im unteren Mikrogrammbereich verhindern Messunsicherheiten die exakte Überwachung. Andere Methoden mit einer besseren zeitlichen Auflösung werden derzeit entwickelt, um niedrige Ammoniakkonzentrationen und die potenzielle Überschreitung kritischer Werte genau zu bestimmen. Die differentielle optische Absorptionsspektroskopie (DOAS) und Cavity Ring-Down Sensoren (CRDS) sind vielversprechende neue Technologien und werden derzeit an einigen Standorten für die zeitaufgelöste, kontinuierliche Online-Überwachung von Ammoniak in den Niederlanden, Großbritannien, Deutschland und der Schweiz eingesetzt.

Im Gegensatz zu den Ergebnissen nationaler Emissionsinventare und den Berechnungen mit Stickstoffdepositionsmodellen hat die Überwachung der Ammoniakkonzentrationen in den oben genannten Ländern gezeigt, dass die Konzentrationen in den letzten zehn Jahren keine Abwärtstrends gezeigt haben. In den meisten Ländern steigen die Ammoniakkonzentrationen leicht an, und es werden dafür zwei Gründe verantwortlich gemacht. Erstens führt die starke Reduzierung der SO_2-Emissionen in den letzten drei Jahrzehnten zu einer geringeren Bildung von Sekundärpartikeln $(NH_4)_2SO_4$, wodurch sich die Verweildauer und der Langstreckentransport von Ammoniak in der Atmosphäre erhöht. Zweitens werden auch die erhöhten Temperaturen aufgrund des Klimawandels für höhere Verdunstungsraten von Ammoniak z. B. aus Wirtschaftsdünger, Pflanzenoberflächen, Böden und Gewässern verantwortlich gemacht. Nicht zuletzt können höhere Konzentrationen auch auf zuvor unterschätzte Emissionen zurückzuführen sein, z. B. aufgrund von Unsicherheiten bei Emissionsfaktoren oder Aktivitätsdaten in der Landwirtschaft, aus Abwasseraufbereitungsanlagen und Biogasanlagen sowie durch den verstärkten Einsatz von Dieselabgasflüssigkeiten (DEF, AdBlue). Wir verweisen auf Referenzen, die zeigen, dass Ammoniakemissionen von Dieselfahrzeugen in den letzten Jahren eine größere Rolle gespielt haben. Wir weisen hier auch auf mögliche zukünftige Emissionen aus Ammoniak-Lagerbehältern hin, die in naher Zukunft zur Produktion von Wasserstoff eingesetzt werden.

Um festzustellen, ob und wie die vorgeschlagenen UN-ECE-Grenzwerte für Ammoniak in Europa angewendet werden, hatten wir am Anfang des Projekts eine Umfrage unter 57 Experten aus 14 Ländern durchgeführt. Es gab zehn Antworten aus sechs Ländern, aber Informationen darüber, wie mit Ammoniak auf lokaler und regionaler Ebene umgegangen wird, waren sehr spärlich. Die Gesetzgebung und Kontrolle von Ammoniak sind vor allem in den Niederlanden, Dänemark, Deutschland und dem Vereinigten Königreich weit entwickelt. Keiner der Befragten wusste von aktuellen wissenschaftlichen Arbeiten, die experimentell die Wirkung von Ammoniak in Begasungskammern untersuchten. Der Kontakt zum CEH Edinburgh lieferte jedoch einige neue Publikationen aus dem weltweit einzigartigen Whim Bog Experiment und wir erhielten einige aktuelle Informationen über Projekte in Großbritannien und Übersee.

Im AP2 wurden mit gezielten Experimenten neue Informationen über die Reaktion stickstoffempfindlicher Pflanzentaxa auf Ammoniak generiert. Der Ansatz sah ein kontrolliertes Begasungssystem vor, mittels dessen fünf Ammoniakbehandlungen im Bereich von 0 bis 10 $\mu g\ m^{-3}$ erzeugt werden sollte. Es ging also um niedrige Konzentrationen, wie sie in der Umwelt vorkommen, bei denen sich mitunter chronische Reaktionen in empfindlichen Pflanzengemeinschaften entwickeln. Die Experimente erforderten die Verwendung von N-empfindlichen gefährdeten Pflanzenarten und zwei Versuchssaisons.

Der klassische ökotoxikologische Ansatz sollte in der Lage sein, Dosis-Wirkungs-Beziehungen zwischen Ammoniakkonzentrationen (Exposition) und relevanten Endpunkten, d.h. biologischen

Effekten wie Wachstumsreaktionen, abzuleiten. Es wurden umfangreiche Überlegungen über die Ammoniakquelle und die Expositionsmethode für den Begasungsversuch angestellt. Auf die Verwendung von technischem (gasförmigem) Ammoniak, das direkt aus Druckbehältern zugeführt wird, wurde verzichtet, da die versehentliche Freisetzung aus solchen in geschlossenen Umgebungen ein ernstes Gesundheitsrisiko für die im Gewächshaus arbeitenden Personen darstellt. Gleichzeitig können zwischendurch auftretende höhere Konzentrationen die Aluminiumstrukturen der Gewächshauskompartimente schädigen. Wir arbeiteten daher mit verdünnten (harmlosen) chemischen Lösungen, in der Form von wässrigem Ammoniak (NH_4OH), was eine schonende und vollständige Verdampfung von Ammoniak im Laufe der Zeit ermöglichte. Die genaue Menge an Ammoniak (in der Flüssigkeit) sowie das Volumen und die Belüftungsraten der Kammern ermöglichten es uns, die mittleren Konzentrationen zu berechnen und einzustellen. Insgesamt wurden fünf Konzentrationen im Bereich von 0 bis 10 µg m^{-3} angestrebt.

Im ersten Jahr, der Saison 2020, wurden die Experimente in fünf Kompartimenten der neuen Gewächshausanlage der Universität Hohenheim, dem Phytotechnikum (PHT), durchgeführt. Für die Überwachung der realisierten Ammoniakkonzentrationen setzten wir Radiello® Passivsammler ein, da sie weit verbreitet sind und eine radiale Geometrie des absorbierenden Materials und somit eine hohe Sammelrate aufweisen. Die Probenahmeintervalle konnten dadurch auf eine Woche statt auf zwei oder vier Wochen wie bei anderen Sammlertypen verkürzt werden. Eine schonende und zeitgesteuerte Verdampfung von verdünnten Ammoniak-Lösungen wurde durchgeführt, um niedrige Konzentrationen in vier Gewächshauskompartimenten einzustellen. Die fünfte Gewächshauseinheit wurde als Kontrolle verwendet, bei der kein Ammoniak in die Kammer gegeben wurde. Die Behandlungen wurden alle zwei Wochen zwischen den vier begasten Kompartimenten rotiert, um Platzierungseffekte zu vermeiden.

Für die Versorgung der vier Kammern mit flüssigem Ammoniak verwendeten wir eine mehrkanalige Schlauchpumpe und lange Teflonschläuche®. In jeder der Kammern wurden die über die Schläuche zugeleiteten Tropfen flüssigen Ammoniaks mit Ventilatoren verdampft. Für die Pflanzenstudie wurden die Pflanzen fünf Ammoniakkonzentrationen ausgesetzt. Darüber hinaus wurde die Hälfte der Pflanzen einer Dürrebehandlung unterzogen, um die Wechselwirkungen zwischen Ammoniak und der Klimawandelkomponente Trockenheit experimentell zu untersuchen.

Die ausgewählten Pflanzenarten wurden als Saatgut von verschiedenen Lieferanten geliefert und bereits im Spätsommer 2019 ausgesät. *Koeleria glauca* (Schrad.) DC. (Blaugras), *Antennaria dioica* (L.) Gaertn. (Katzenpfötchen), *Dianthus deltoides* L. (Heidenelke), *Carex arenaria* L. (Sand-Segge) und *Arnica montana* L. (Arnika) wurden aus kommerziell erhältlichem Keimgut gezogen, während wir von letzterer Art auch Samen aus *Nardus*-Grünland der Hohen Rhön besorgen konnten. Da Arnika, eine Flaggschiffart sauren montanen Grünlands, aus zwei unterschiedlichen genetischen Herkünften herangezogen werden konnte, lies sich auch der Frage nachgehen, ob zwei Samenursprünge eine unterschiedliche Reaktion auf Ammoniak zeigen würden. Neben diesen fünf aus Samen gezogenen Arten vermehrten wir Ausläufer von *Molinia caerulea* (L.) Moench (Pfeifengras) und kauften vorkultivierte Pflanzen von *Pulsatilla vulgaris* Mill. (Küchenschelle). Da wir zehn Replikate pro Ammoniakbehandlung und Trockenunterbehandlung verwendeten, brauchten wir 100 Pflanzenindividuen pro Art. Die Pflanzen wurden in 1L-Plastiktöpfen kultiviert und das verwendete Substrat bestand aus einem Standard-Erde- und Bodenmaterial, das von natürlichen Standorten stammte, an denen die von uns ausgewählten Pflanzenarten wachsen. Zu Beginn des Experiments wurde bei allen Pflanzen eine Düngung mit ^{15}N-markiertem NH_4NO_3 durchgeführt, aber die stabilen Isotopen wurden später nur in Arnika und *Molinia* untersucht, da die eine negativ und die andere Art positiv auf die unterschiedlichen Ammoniakkonzentrationen reagieren sollten.

Die Gewächshausbegasungsexperimente liefen in der Vegetationsphase des Jahres 2020, begannen also im Mai 2020 und dauerten bis Oktober 2020. Die Pumpe und die Schläuche für die Ammoniakexposition funktionierten während der fünfmonatigen Begasungsstudie ohne Ausfall, und die resultierenden Ammoniakkonzentrationen und Durchschnittstemperaturen wurden während der gesamten Studie in jeder der fünf Kammern überwacht. Während die Ammoniakdaten aufgrund der Verwendung von Passivsammlern eine wöchentliche Auflösung aufwiesen, wurden Temperatur und Luftfeuchtigkeit auf einer 1h-Basis aufgezeichnet.

Die Ammoniakkonzentrationen variierten während des Experiments und betrugen in der Kontrolle durchschnittlich 5,2 bis 17,6 µg m^{-3} in der höchsten Behandlung. Die Pflanzen waren somit etwas höheren Konzentrationen ausgesetzt als ursprünglich vorgesehen. Gleichzeitig waren die gemessenen Temperaturen in den Gewächshauskompartimenten um 6,7 °C höher als im Freien und Spitzenwerte von über 40 °C erforderten eine regelmäßigere Wasserversorgung. Dennoch konnten wir eine Trockenbehandlung mit durchschnittlich 33% weniger Wasser für die Hälfte der Pflanzen realisieren. Trotz des Insektenbefalls an der Arnika entwickelten alle Arten gesunde Pflanzen und nur wenige Individuen starben während des Gewächshausexperiments. Wir sind daher sicher, dass die Ergebnisse unseres Experiments im Allgemeinen zuverlässig waren, aber es muss dennoch angemerkt werden, dass die Phänologie verändert zu sein schien, da die meisten Pflanzen aufgrund fehlender Vernalisation (Blühinduktion bei Pflanzen nach einer längeren Kälteperiode) nicht in das Stadium der Blüte eintraten.

Die Sprossbiomasse wurde bei einer Zwischen- und einer Endernte betrachtet. Alle Arten zeigten in beiden Ernten einen signifikanten Trockenheitseffekt auf die Sprossbiomasse, während die Auswirkungen von Ammoniak nur bei wenigen Arten in der Zwischen- und Endernte des ersten Jahres signifikant waren. Varianzanalysen und *post-hoc*-Mehrfachvergleiche (Tukey-Tests) bestätigten viele signifikante Unterschiede zwischen den Behandlungen, aber wir beobachteten keine linearen Reaktionen von der niedrigsten zur höchsten Konzentration. Dies mag darauf zurückzuführen sein, dass der Bereich der realisierten Konzentrationen keine großen Unterschiede im unteren und mittleren Bereich aufwies, so dass es nicht möglich war, Dosis-Wirkungs-Kurven abzuleiten. Wir berechneten daher sogenannte Response Ratios (RR), d.h. die Biomasse, die in den höchsten (17,6 µg m^{-3}) im Verhältnis zu den niedrigsten (5,2 µg m^{-3}) Ammoniakkonzentrationen produziert wurde, und schauten vorrangig auf Wachstumsreaktionen von mehr als 20%.

Interessanterweise fehlten Wachstumsstimulationen von über 20% im ersten Jahr, während Wachstumsrückgänge aufgrund des erhöhten Ammoniaks bei den Trockenbehandlungen von *Antennaria dioica*, *Koeleria glauca*, *Carex arenaria* und *Dianthus deltoides* sowohl bei geschnittenen als auch bei ungeschnittenen Pflanzen beobachtet wurden. In der produktiveren Herkunft von *Arnica montana* produzierten die gut bewässerten, geschnittenen Pflanzen bei der erhöhten Ammoniakbehandlung weniger Biomasse.

Eine zweite Versuchssaison folgte der Gewächshausstudie, um die Auswirkungen von Ammoniak auf die Wuchsleistung mehrjähriger Pflanzen zu untersuchen, die zuvor Kombinationen aus Trockenheit und Schneiden (Mähen) ausgesetzt waren. Um die Pflanzen nährstoffarm zu halten, wurden die Töpfe im Frühjahr 2021 nicht gedüngt. Da die Pflanzen im ersten Jahr im Gewächshaus extremen, unnatürlichen Temperaturen von fast 7°C höher als im Freien ausgesetzt waren, exponierten wir die Pflanzen im zweiten Jahr in einem Freilandversuch. Dazu etablierten wir im Lee des Universitätseigenen "Unteren Lindenhofs" fünf Stationen, die ein natürliches Klima und einen für landwirtschaftliche Anlagen typischen Ammoniakgradienten repräsentieren.

Die saisonalen Ammoniakkonzentrationen auf dem Feld reichten von 1,8 am weitesten entfernt bis 9,4 µg m^{-3} in der Nähe der Ställe und repräsentierten gut den Konzentrationsbereich, den wir ursprünglich anstrebten. Die drei mittleren Konzentrationen lagen jedoch nur zwischen 2 und 4,7 µg m^{-3} und es wurden wie im Vorjahr keine linearen Beziehungen des Pflanzenwachstums zu den Ammoniakkonzentrationen gefunden. Dennoch war das Klima mehr oder weniger vergleichbar mit der Langzeitsituation und die manuelle Bewässerung zu trockeneren Zeiten sorgte für eine normale Entwicklung der Pflanzen.

Neben den mehrjährigen Pflanzenarten wurden auch fünf vorkultivierte einjährige Pflanzenarten an den fünf Stationen ausgebracht, nämlich *Iberis amara* (Schleifenblume), *Trifolium arvense* (Hasenklee), *Bromus hordeaceus* (Weiche Trespe), *Erodium cicutarium* (Reiherschnabel) und die seltene *Legousia speculum-veneris* (Venusspiegel). Die Pflanzenauswahl basierte auf funktionellen Eigenschaften, taxonomischen Kriterien und der Bevorzugung der Arten stickstoffarmer Lebensräume. Im zweiten Jahr der Experimente wurden weder die Stauden noch die Einjährigen einer Trockenbehandlung unterzogen und die Ernten wurden nur einmal am Ende der Saison durchgeführt. Pflanzenexpositionen und Ernten der 13 Pflanzenarten unterschieden sich jedoch je nach Phänologie. Am Ende der Saison konnte die Sprossbiomasse mit den verschiedenen Ammoniakkonzentrationen in Relation gesetzt werden, die an den fünf Stationen für die Dauer der Exposition registriert wurden.

Um die Wachstumsreaktionen mehrjähriger Arten auf Ammoniak zu verstehen, ist es wichtig, ihre unterschiedlichen ökologischen und lebensgeschichtlichen Merkmale zu berücksichtigen. Während die drei Arten *Koeleria glauca*, *Dianthus deltoides* und *Pulsatilla vulgaris* in der zweiten Saison >30% weniger Biomasse produzierten, konnten *Antennaria diocia*, *Carex arenaria* und *Molinia caerulea* >40% mehr Sprossmasse produzieren, obwohl in der zweiten Saison kein zusätzlicher Dünger verabreicht wurde.

Die angeborene, d.h. genetisch fixierte Fähigkeit, Ressourcen für das Wachstum unter begrenzten Bedingungen zu erwerben, wird einen starken Einfluss auf die langfristige Leistungsfähigkeit von Pflanzenarten haben. Der stärkste Anstieg der Biomasse, obwohl in absoluten Zahlen nur die drittproduktivste Art, wurde bei *Molinia caerulea* beobachtet, einer Grasart, die eine breite ökologische Amplitude aufweist und in Heidegebieten in Regionen mit Eutrophierungsproblemen stark zugenommen hat. Ammoniak erhöhte die Sprossmasse in jeder der Unterbehandlungen signifikant, unabhängig davon, ob die Pflanzen unter trockenen Bedingungen gehalten oder in der vorherigen Saison einem Zwischenschnitt unterzogen worden waren. Wir kommen zu dem Schluss, dass der potenzielle Stress, dem *Molinia* durch Mähen und Trockenheit ausgesetzt ist, d.h. die Entfernung oder der nicht erfolgte Aufbau von Ressourcen im Vorjahr, die Aufnahme und Nutzung von düngendem Ammoniak für die Produktion neuer Biomasse nicht verringert. Tatsächlich produzierten Pflanzen, die in der ersten Saison geschnitten wurden, mehr Sprossbiomasse, wenn sie gleichzeitig trockenen Bedingungen ausgesetzt waren.

Die Ergebnisse legen nahe, dass der Wurzelstock des Horst bildenden Grases als wichtiger Modifikator für Wachstumsreaktionen auf Trockenheit, Biomasseabbau und Ammoniak angesehen werden muss. Wir gehen davon aus, dass die Nährstofffökologie des Süßgrases und die Allokation hoher Anteile an Biomasse zu seinem Wurzelstock es *Molinia caerulea* ermöglicht, ihre Biomasse im zweiten Jahr zu verdoppeln

Ein ähnlicher Anstieg der Sproßmasse vom ersten zum zweiten Jahr wurde bei der Segge *Carex arenaria* beobachtet. Im Gegensatz zu *M. caerulea* konnte jedoch durch erhöhte Ammoniakkonzentration keine Wachstumsstimulierung beobachtet werden. In beiden Jahren zeigten die ungeschnittenen Pflanzen aus der Trockenbehandlung eine verminderte Biomasse unter erhöhtem Ammoniak.

Wir sind uns über die zugrunde liegenden ökologischen Mechanismen nicht völlig im Klaren, aber vermuten, dass die Ausläufer und Horst bildende Grasart relativ nährstoffarme Bedingungen benötigt, um sich vegetativ zu vermehren. Ein entgegengesetztes Verhalten könnte der Grund für die reduzierte Biomasse von *Koeleria glauca*, *Dianthus deltoides* und *Pulsatilla vulgaris* sein. Da die Sproßmasse der drei Arten im zweiten Jahr deutlich geringer war als im ersten Jahr, können wir davon ausgehen, dass diese Arten bei geringer Nährstoffversorgung nicht langlebig sein werden. Es ist jedoch möglich, dass andere Nährstoffe wie P, K und Ca neben Stickstoff limitierend wirken. Man muss auch darauf hinweisen, dass einige Pflanzenarten NO_3-N gegenüber NH_4-N bevorzugen und Ammoniak nicht gut verstoffwechseln.

Widersprüchliche Ergebnisse wurden bei den beiden Kompositenarten *Antennaria dioica* und *Arnica montana* gefunden. Während das Katzenpfötchen bei der Exposition gegenüber erhöhtem Ammoniak die Sproßmasse von ungeschnittenen Pflanzen verringerte, nahm die Sprossmasse in der kommerziellen Linie von *Arnica* tendenziell zu. Viele Individuen der empfindlichen Arnika starben jedoch während der Gewächshausexperimente, so dass wir uns über die Repräsentativität der Ergebnisse nicht sicher sind.

Während die Ergebnisse der Wachstumsanalysen bei den Stauden zumeist gegenläufig waren, zeigten drei von fünf Einjährigen eine Zunahme der Sprossmasse, wenn sie in der Nähe der Ställe exponiert wurden. Offensichtlich konnten sie durch Ammoniak ihr Wachstum steigern, während die langsam wachsende *Legousia speculum-veneris* keine klaren Reaktionen zeigte und *Trifolium arvense* eine 32%ige Reduktion der Sprossmasse.

Wir gehen daher davon aus, dass das Wachstum kurzlebiger Arten, der so genannten Ruderalflora, von erhöhten Ammoniakkonzentrationen gesteigert werden kann. Die negative Reaktion von Hasenklee deutet darauf hin, dass N-fixierende Arten unterschiedlich auf die Ammoniakzufuhr reagieren könnten. Wir schlagen daher vor, in Zukunft weitere Leguminosenarten zu untersuchen.

Um die Aufnahme und Remobilisierung von Stickstoff nachvollziehen zu können, wurden alle Pflanzen vor Beginn des Begasungsversuchs leicht mit einer mit ^{15}N angereicherten Nährlösung gedüngt. Es wurde angenommen, dass die Aufnahme von Stickstoff aus Ammoniak – verflüchtigt aus flüssigem Ammoniak enthält es höhere Anteile des leichteren Isotops – im Laufe der Zeit zu einer ^{15}N-Verdünnung führen würde. Wir führten Analysen der ^{15}N- und Stickstoffgehalte der verschiedenen Sprossfraktionen (seneszente Blätter und Stängel) der beiden Ursprünge von *Arnica montana* und von *Molinia caerulea* durch. Da Wurzeln die größten Organe von Gräsern sind, analysierten wir auch die N-Konzentrationen und Isotope im Pfeifengras.

Die Verdünnung von ^{15}N im Laufe der Zeit, d.h. die Absenkung des δ Signals, war bei seneszenten Blattfraktionen beider Arnika-Ursprünge, die im ersten Jahr gesammelt wurden, gut sichtbar. Die Signaturen sanken vom eingestellten Wert von 100 in Blättern, die zuerst abstarben, auf 30 in der Fraktion, die erst im Winter verwelkte. Anfangs war die ^{15}N-Verdünnung in den Pflanzen, die unter trockeneren Bedingungen angebaut wurden, weniger ausgeprägt. Am Ende der Saison war dieser Unterschied aber nicht mehr bemerkbar.

Die Ergebnisse bestätigen, dass Pflanzen, deren Wachstumsbedingungen durch Stress verschlechtert werden, zunächst die vorhandenen Ressourcen nutzen und erst später nennenswerte Mengen an atmosphärischem Ammoniak aufnehmen. Während dieser Effekt in den seneszenten Blättern zu sehen war, die in der ersten Saison abgeworfen wurden, unterschieden sich im zweiten Jahr die Isotopensignaturen zwischen Pflanzen aus der trockenen und der gut bewässerten Behandlung nicht mehr. Dennoch unterschieden sich die Isotopensignaturen zwischen den beiden Arnika-Populationen, wobei die produktivere kommerzielle Herkunft eine stärkere ^{15}N-Verdünnung zeigte als die langsamer wachsenden Pflanzen aus der Wildpopulation. Beim Vergleich der Stickstoffkonzentrationen in den Pflanzenfraktionen und des Gesamtstickstoffs, der in der Sprossmasse gebunden war, die unter unterschiedlicher Exposition gegenüber Ammoniak gewachsen war, konnten wir keine Auswirkungen von Ammoniak feststellen. Die Pflanzen aus der Wildpopulation hatten jedoch nur die Hälfte des Stickstoffs auf Topfbasis aufgenommen und eingelagert als die aus dem schnell wachsenden kommerziellen Samenursprung der Arnika.

Ein Großteil der Aufnahme von NH_4^+ aus deponiertem gasförmigem Ammoniak geschieht über die Pflanzenoberfläche und es ist wahrscheinlich, dass aufrechte Blätter und Stängel von Gräsern besser in der Lage sind, Ammonium aus der Atmosphäre auszukämmen als flachblättrige stämmige Kräuter. Tatsächlich zeigten zwei der Gräser, die wir in unserer Studie verwendeten, ein stärkeres Wachstum in der Nähe des Hofes als Folge der höheren Ammoniakkonzentrationen. Obwohl die Spross-N-Konzentrationen in *Molinia caerulea* unabhängig von der Entfernung zum Betrieb allesamt unter 0,6% blieben, nahm die Stickstoffmasse, die auf Topfbasis vorhanden war, entlang des Gradienten mit zunehmender Entfernung ab. Es muss aber beachtet werden, dass Pflanzen, die in der vorherigen Saison geschnitten worden waren, eine geringere Stickstoffmenge pro Topf hatten als diejenigen, die keiner Zwischenernte unterzogen wurden. Obwohl wir eine stärkere ^{15}N-Verdünnung in den Trieben von geschnittenen und ungeschnittenen Pflanzen sahen, die in der Nähe des Stalls wuchsen, beobachteten wir keine lineare Beziehung zu den mittleren Ammoniakkonzentrationen.

Interessanterweise war die ^{15}N-Verdünnung in den Wurzeln geringer, was bestätigt, dass ein Großteil des in der Wurzelmasse fixierten Stickstoffs noch aus der Düngung stammte, die zu Beginn des Experiments ein Jahr zuvor angewendet wurde. Unsere Experimente basierten auf einer Studie, in der nur eine Pflanze pro Topf gezogen wurde und sind daher nicht unbedingt repräsentativ für Pflanzen, die in Koexistenz mit anderen Individuen und Pflanzenarten wachsen. Obwohl es wichtig ist, über taxonomische Unterschiede in der Empfindlichkeit verschiedener Pflanzenarten Bescheid zu wissen, wird es notwendig sein, ammoniakbedingte Veränderungen in Pflanzengemeinschaften an natürlichen Standorten anzugehen.

In **AP3** wurde eine Machbarkeitsstudie für die Untersuchung von Ammoniak in etablierter Vegetation durchgeführt. Die interessantesten Lebensräume und Ökosysteme sind diejenigen, die sich unter nährstoffarmen Bedingungen entwickelt haben, z.B. Heiden, Moore und Wiesen auf flachen sauren Substraten. Da Ammoniak die schnell wachsenden produktiven Arten begünstigen dürfte, werden kleinere Kräuter von den dominanten oft Grasartigen überholt. Neben Feldbegasungsexperimenten befassten wir uns auch mit der Untersuchung von Pflanzen in Ammoniakgradienten im Lee von Emittenten und der möglichen Verwendung von Bioindikatoren, z.B. in Citizen Science-Experimenten.

Während Open-Top-Kammern (OTC) als Standardtechnologie verwendet wurde, um die Auswirkungen der Luftverschmutzung (SO_2, O_3 und NH_3) in etablierten Vegetationen zu untersuchen, ist weltweit nur ein kammerloses Feldmanipulationsexperiment etabliert worden, das einen quantifizierten Ammoniakkonzentrationsgradienten (Depositionsgradienten) in einem ombrotrophen Moor unter natürlichem Klima erzeugt. Experimente wurden 2002 im schottischen Whim Bog begonnen und der Standort ist nun anerkannter Studienort des Horizon 2020 EU-Programms eLTER - European Long-term Ecosystem Research. Vergleichbare Begasungssysteme hat der Betreiber (CEH Edinburgh) kürzlich in Wäldern in Schottland und Sri Lanka aufgebaut.

Trotz der Unzulänglichkeiten der Freisetzung von reinem Ammoniak in eine etablierte Vegetation und der teils sehr hohen Konzentrationen und NHx-Depositionen in der Nähe des Auslasses ist das System wahrscheinlich die derzeit beste verfügbare Technologie. Anstatt unverdünntes Ammoniak durch aktive Begasung in die Vegetation freizusetzen, könnten aber auch Freilandexpositionssysteme entwickelt werden, die auf der passiven und schonenden Verflüchtigung von verdünntem flüssigem Ammoniak aus porösen Schläuchen (Perlschläuche) beruhen. Zeitgesteuerte Pumpen könnten verwendet werden, um die Flüssigkeit zuzuführen und Konzentrationsgradienten zu erzeugen, aber ein Dach und ein Trog müssen die Rohre vor Regen und vor dem Austreten / Auslaufen von flüssigem Ammoniak in die Parzellen schützen. Mit einer entsprechend guten Finanzierung der Ausrüstung, der Betriebskosten und des technischen und wissenschaftlichen Personals wird es möglich sein, ein ähnliches oder verbessertes System wie das vom CEH entwickelte einzurichten. Es wäre wünschenswert, zwei Systeme, je eines in einem Moor und einem Niedermoor im Norden bzw. Süden Deutschlands und zwei weitere Anlagen in saurem und kalkhaltigem Grünland zu haben. Wälder müssen von Ammoniakbegasungsexperimenten ausgeschlossen werden, aber wir empfehlen, vier deutsche Standorte mit Ammoniakexperimenten in niedrig wachsender Vegetation einzurichten.

Mögliche Standorte für die experimentelle Ammoniakexposition sollten dort in Betracht gezogen werden, wo bereits langfristige ökologische Forschung durchgeführt wurde bzw. noch durchgeführt wird. Idealerweise sollte eine Forschungsstation mit Stromversorgung in der unmittelbaren Nähe liegen. Es werden im vorliegenden Bericht verschiedene Netzwerke identifiziert, die sich mit der Erforschung des globalen Wandels in Vegetationen befassen, z.B. die Untersuchung bi-direktionaler Flüsse klimarelevanter Gase und die Wiederherstellung von Mooren. Die atmosphärische Komponente Ammoniak und die Auswirkungen der Eutrophierung könnten in einige dieser laufenden Programme integriert werden.

Schließlich befassten wir uns auch mit Gradientenstudien im Lee großer Tierhaltungsanlagen. Um mögliche schädliche Auswirkungen auf die Natur zu begrenzen, werden große Ställe mit vielen Tieren und vermutlich hohen Ammoniakemissionen nur an Standorten außerhalb stickstoffempfindlicher Bereiche zugelassen. Es wird daher an solchen Orten nicht möglich sein, die Auswirkungen von Ammoniak auf die etablierte natürliche Vegetation zu erforschen. Wir schlagen daher vor, einen ähnlichen Ansatz wie in WP2 anzuwenden. Vorgezogene N-empfindliche Pflanzenarten können als Bioindikatoren entlang des Ammoniakgradienten im Windschatten großer Ställe exponiert werden. Neben einjährigen Pflanzenarten, die in unserem Experiment positive Wachstumsreaktionen zeigten, wäre eine vielversprechende Art auch das Gras *Molinia caerulea*, das im Versuch ebenfalls ammoniakgetriebene Wachstumsveränderungen zeigte. Da wir im niedrigen Konzentrationsbereich zwischen 2 und 10 µg m^{-3} gearbeitet haben, in dem keine nachteiligen Auswirkungen auf die Pflanzen beobachtet wurden, wird es interessant sein, höhere subakute Konzentrationen von > 30 µg m^{-3} einzubeziehen, die in der Umgebung größerer Ställe typisch sind. Es kann durchaus sein, dass solche Konzentrationen höhere Pflanzen bereits physisch schädigen können. Neben der Verwendung von Einzelpflanzen empfehlen wir die Nutzung von Artenmischungen, die tiefere Einblicke in die ammoniakgetriebenen Veränderungen der Wettbewerbsfähigkeit und langfristige Veränderungen in der Vegetation geben könnten. Die Verwendung einer Gras-Klee-Mischung, z. B. *Molinia caerulea*, die mit einer *Trifolium*-Art zusammenwächst, als Bioindikator könnte eine Kombination einer Art sein, die eine wachstumssteigernde Reaktion zeigt, mit einer Art, bei der sich geänderte Ammoniakkonzentrationen nicht auf das Wachstum auswirken.

Nach der Erprobung verschiedener Pflanzenkombinationen im Lee von Ställen kann der beste Ansatz auf andere Untersuchungsstandorte und Programme übertragen werden. Wir empfehlen, neue Bioindikationsansätze zu entwickeln, die z.B. in nationalen Citizen Science-Projekten und internationalen Kooperationsprojekten wie ICP-Vegetation eingesetzt werden können.

1 Project objectives and milestones

As part of the departmental research plan (ReFoPlan) of the Federal Environment Agency (UBA), a project with the title "***Experimental and literature review of internationally proposed critical levels for ammonia to protect the vegetation***" was tendered in the end of the year 2018. In April of the following year, the Institute of Landscape and Plant Ecology of the University of Hohenheim (UHOH 320b, Stuttgart, Germany) was awarded the contract and soon after the recruitment of additional staff began to work on the topic. The proposed project consisted of four work packages,

- literature survey (**WP1)**
- ammonia fumigation experiment under controlled conditions (**WP2)**
- feasibility study to design and prepare field fumigation and transect experiments **(WP3**)
- preparation and follow-up reporting of an expert workshop **(WP4)**

An overview of the original timing of the project as well as the deliverables from the four WPs is given in Figure 1. Besides the WPs 1 to 3, the results of which were communicated in seven unpublished interim reports, the contractor in WP4 should organise an international expert workshop in the spring of the final year to present and discuss the results from the project. In order to prepare the meeting, which was held in Dessau on 28 and 29 March 2022, the contractor and the contracting authority jointly set up an agenda, invited experts for presentations and widely distributed the announcement and invitation to the meeting. A background document (BD) had to be authored as an important deliverable before and a summary needed to be compiled after the workshop. The proceedings include the updated BD and the extended abstracts of in total 19 presentations, which were being held during the meeting (Franzaring and Kösler, 2023.). The document is a stand-alone project output and will serve as a valuable backup reference for international policy makers, who are involved in the regulation and monitoring of potentially adverse effects of atmospheric ammonia. The literature review presented in UBA-Texte (31/2023)) is a short version of the chapter 2.2.

Figure 1: Work packages, original timing and deliverables of the project

Work Packages 1 to 4 and deliverables

Source: Own illustration, University of Hohenheim.

Conducting the project was affected by the Covid19 pandemic in several ways, but at no time the proper and timely progress of the works were at risk. While inside the offices and the greenhouses used for the experiments, the corona regulations and social distancing were always adhered to, most of the project meetings and regular communication between the contracting partners were done via video platforms, i.e. webex and zoom. Since corona regulations were lifted towards the end of the project, the Dessau expert workshop (Figure 1) could be held as a hybrid meeting. In fact, we assume that via the online format we were able to reach a much wider audience since over registered 100 participants joined and discussed online.

In addition to mail contacts and to the Dessau workshop, we also presented and discussed our project outcomes on two other international conferences (8th INI Global Nitrogen Conference, May 30th 2021 and the conference on Frontiers in Experimental Research on Changing Environments, June 7th to 9th 2022) as well as on two national workshops (Hildesheim, 5th November 2019 and 4th November 2021). We are happy to acknowledge the numerous comments, references and textual additions we received from several colleagues after the Dessau Workshop. Much of the information we received there, was also fed into the above-mentioned proceedings document of the workshop (UBA-Texte (31/2023)) and into the present chapter 2.2 with some identical abstracts and tables. We would like to acknowledge especially the input of Matthew Jones (CEH) and Alexander Moravek (UBA).

Nevertheless, during the three years, we had to slightly modify our plans due to experimental reasons. These changes were done after intense discussions, the conceptual input and feedback and in consent with the contracting authority. Major modifications involved WP2, in which pre-cultivated plant species should be fumigated with ammonia in greenhouse compartments for two seasons. We decided to not continue with that approach after the first summer since the temperatures were too high in the greenhouse and created an unnatural growth climate. Instead, we moved from a chamber-based fumigation to a transect study in the second season (2021), involving a natural ammonia gradient in the lee of the university farm. With these changes we were also able to get practical input to WP3, which should deal with a feasibility study to design and prepare field fumigation and transect experiments.

The present report gives a full account of the project. For a comprehensive chronicle of the project milestones, discussions and details on the development of technical approaches, we refer to the seven unpublished interim reports, which were approved each time prior to the programmed instalments. Both, the interim and final reports will be the basis for scientific papers we hope to be writing during the following year. An extensive literature review manuscript of over 30 pages had already been prepared in December 2021 and was fed in a shortened version into the above-mentioned background document. Next chapter, however, will give a longer version of our literature review and the means how we collected data and information unavailable in the public literature. With this review chapter, our own experimental studies and the final chapter of this report, we strive to raise more awareness on planning field-based studies in the future.

2 Review of critical levels for ammonia (WP1)

In the extensive literature study, i.e. WP1 of the tender and for the later preparation of the expert workshop, the background document (BD) and conference proceedings (WP4), throughout the whole project we continuously kept on collating and evaluating important peer-reviewed references and grey literature that had been published before and after the year 2009 describing scientific findings on the potentially adverse effects of ammonia on plants and ecosystems. Special focus was laid on:

- experimental studies which present dose-response relationships for clear physiological effects (in controlled environments) and responses that were derived from ecological field-based studies (e.g. in the lee of emitters)
- regulations and enforcement aids from different countries that are applying critical levels in the ecological impact assessment
- information on ammonia monitoring programs using passive samplers and real-time monitoring devices.

For latter two practice-related objectives, we already in the very beginning of the project consulted experts from various European countries to get an impression on the different approaches used in the ecological risk assessment, e.g. for plans and projects in Natura2000 areas and the overall willingness to tackle the eventual exceedance of critical levels of ammonia in different regions. Many of these experts, which we contacted via a steadily growing mailing list later also joined the international workshop in Dessau (28/29 March 2022) to keep up and to update the discussion.

As an entry point, the text box in the following chapter gives a short overview of the history of ammonia research and mentions the two UNECE workshops (Egham 1992 and Leith 2006), at which critical levels for ammonia were suggested by experts dealing with the adverse ecological effects of the gas. Our project WP1 had the objective to mainly sum up information that was becoming available after 2009, focusing on the question whether critical levels for ammonia need to be modified in the future.

2.1 Survey among experts

In our first call to assist with the supply of information relevant to updating critical levels for ammonia, 57 people from 14 European countries were contacted by E-mail. We asked for following information:

- What are the general recommendations for action in your reach on evaluating/reducing ammonia emissions from intensive livestock stables, biogas plants, major roads (traffic emits ammonia as well) and waste water treatment facilities? These may be based on the use of dispersion models and air pollution regulations.
- How do you monitor ammonia and its adverse effects? Do you use passive samplers and/or vegetation surveys in the vicinity of ammonia emitting facilities (stables, biogas plants, roads, WWTPs). Who is planning and coordinating those programmes?
- Do you know of new experimental findings in the lab and in the field to test ammonia effects?

Following our first online survey, we received ten replies from six countries. We were hinted to an important report by Reinds et al. (2019), who provided a good summary on policies and practises in France, Germany, the United Kingdom, the Netherlands and Denmark. All countries are currently applying or developing biodiversity-based critical loads (Bobbink et al. 2011; Hettelingh et al. 2014), but critical levels are only used in the UK, Denmark and Germany within the framework of licensing new installations. Adopting low reference concentrations for ammonia e.g. in the assessment of plans and projects in and outside of Natura2000 habitats would certainly put high pressures on the agricultural and traffic sectors.

We were also hinted to a new guideline to evaluate adverse nitrogen inputs from traffic and livestock farming (LAI & LANA 2019), which was based on the project by Balla et al. (2013). The document had jointly been elaborated by federal and state working groups on emission control and nature protection and was based on recommendations from a research project on traffic-related nitrogen deposition. Another recent study from Germany was mentioned, that established critical levels for ammonia for all Natura2000 habitat types in Germany (StickstoffBW 2019, Ssymank et al. 2022, 2023). These values are being cross-filed against empirical critical loads (empirical CLO and SMB-CLO) and Ellenberg N-values for the characteristic species. The working groups suggest that using these area-based critical levels will be much easier than using critical loads, which will need numerous local modifications in the models. Information from Germany came also from colleagues from four of the larger Federal States. Obviously, levels of activity differ in the regions due to the different importance of the livestock sector. In Lower Saxony, a state with a very high livestock density in the north-west of Germany, two-day workshops on ammonia are being held every year under the auspices of the Working Environment Authority (Hildesheim). At these occasions, internationally renowned scientists from various European countries present and discuss their novel research and the yearly programmes and presentations are available to registered participants (not to the public) from a password-protected website. Besides organising these annual workshops, several monitoring initiatives have been initiated in Lower Saxony not only in the intensive livestock regions. We also received information from a colleague who operates a lichen monitoring in permanent plots of two pine forest stands. The nitrate concentrations in groundwater and the nitrogen contents in pine needles prove suited indicators for the adverse effects of ammonia deposition in the North-West.

In the south-west of Germany, namely in the state of Baden-Württemberg, the Ministry of the Environment, Climate Protection and the Energy Sector has established a working group on nitrogen (s. above). “StickstoffBW” is coordinated by the State Agency for the Environment, Measurements and Nature Conservation (LUBW) and regularly publishes documents and reports on the monitoring, mapping and assessment of nitrogen deposition. In an ongoing project performed in one of the biggest peat bog complexes in Germany, the Wurzacher Ried, vegetation analyses and ammonia measurements along a deposition gradient have been initiated.

Text Box: History of ammonia research

In the late 1980s, widespread eutrophication, acidification and the novel forest decline were the drivers in Europe and N- America for gathering more scientific evidence on the negative effects of ammonia on nature and ecosystem health. At the same time, the importance of the gas in the generation of secondary particles (PM2.5) and thus, its indirect effects on the human health and the global climate were recognized. Research on the adverse effects of ammonia on plants and vegetation reached a first peak in the 1990s. An overview of some of the numerous reports is given in the photo below. Up to the 1990s and due to its short lifetime, ammonia had not been viewed as an air pollutant, but at the UNECE Workshop on critical levels in Egham, UK, 23–26 March 1992 the gas was dealt with for the first time (Ashmore and Wilson 1994). Following the workshop, the annual critical level of 8 µg m^{-3} became the accepted benchmark for many years. Besides the industrial air pollutants SO_2 and NO_2, ammonia was included in the UNECE Gothenburg Protocol (1999) to abate acidification, eutrophication and ground-level ozone. This was also the beginning of elaborating national emissions ceilings (NEC) for different countries and to prepare guidance documents on how to reduce the emissions. However, until now the gas ammonia was never added to the list of pollutants that need to be monitored in the EU according to the air quality directives (AAQD). For a recent overview of the implications of ammonia derived PM2.5 on human health see Wyer et al. (2022).

Source: Own illustration, University of Hohenheim.

In the early 2000s, research on emission factors, best available technologies (BAT) in animal housing, feeding practice, slurry storage and application technology was intensified. The results were used to establish the European directives on the Integrated Pollution Prevention and Control (IPPC) and later, the Industrial Emissions Directive (IED). At the same time, research on ammonia and nitrogen deposition became an important research topic in Eastern Asia.

A revision of the critical level for ammonia took place at the UNECE Edinburgh Workshop hosted by the Scottish Government at Victoria Quay in Leith, UK 4-6 December 2006. The conference proceedings were edited by Sutton et al. (2009) and the re-assessment of the critical levels has been made by Cape et al. (2009 a, b).

The year 2009 thus marks an important turning point, that is also respected in present project (s. below). The previously valid annual critical level (CLE) of 8 µg m^{-3} NH_3, when expressed as gaseous dry deposition of nitrogen to an ecosystem was found to be less protective than the critical load (CLO) for many European ecosystems and habitats (Cape et al. 2009). At a deposition velocity of 1 cm s^{-1} an NH_3 concentration of 8 µg m^{-3} leads to deposition of 20 kg N ha^{-1} a^{-1}. Field-based experiments and expert judgement suggested that the long-term critical level of ammonia probably needed to be reduced to 3 µg m^{-3} for vascular plants and to 1 µg m^{-3} for N-sensitive lichen and bryophyte species. Underlying information had mostly been based on research from Scotland, i.e. the long-term Whim Bog Experiment and on gradient studies in the lee of poultry farms. In the year 2009, no actual information was available from other, more densely populated and agricultural regions although the background concentrations of the gas are much higher and the long-term effects of ammonia deposition on N-sensitive ecosystems are probably more pronounced in most European countries as compared to Scotland.

Ammonia emissions will have to be reduced significantly in Germany and elsewhere in the EU according to the national emission ceilings laid down by the updated NEC Directive 2016/2284/EU and the 2030 national reduction commitments (NERC). Adopting low reference concentrations for ammonia e.g. in the assessment of plans and projects in and outside of Natura2000 habitats puts high pressures on the agricultural and traffic sectors. We can assume that background levels of ammonia are exceeding the suggested critical levels in the traditional European intensive livestock belts (Northern France, The Po Valley, Flanders, the Netherlands and Northern Germany) and in the metropolitan areas. However, there are currently no methods available to detect and constantly register ammonia concentrations in the lower microgram range, which will probably have to be the backbone of an efficient air quality control of the gas in the future.

Furthermore, LUBW has started to perform ammonia measurements at around 10 sites using Ferm type passive samplers. Another reply came from Bavaria. The state also performs ammonia monitoring at a few locations dominated by agriculture and traffic. Information on ammonia monitoring in Germany was updated on a regular basis and is included in Table 3.

Within the framework of the ICP-Forests programme, ammonia has been determined on some of the German level II plots in the past years, but data have not yet been published. Contacts had been made throughout the project and data were supplied to the contractor. An analysis of these data is presented in Figure 3 in the chapter on ammonia monitoring (chapter 2.2.5).

Only one feedback came from France. Ammonia concentrations are monitored at a few EMEP stations and INRA Rennes coordinates short term projects and campaigns at the field and landscape scale (gradients and transects around agricultural sources). In these projects, passive samplers (Fauvel et al. 2019, mentioned in Table 3) as well as mobile laser measurements are being used, but none of these initiatives is targeted towards vegetation changes in sensitive habitats and the use of critical levels. Overall, the effects of eutrophication on natural ecosystems are not much addressed in France. The same is probably true for Poland, where no information was available on how nitrogen sensitive vegetation is being protected from emissions from the livestock sector.

None of the seven persons contacted in the Netherlands were replying to our initial mails, but later we received more feedback, especially during the preparations of the Dessau workshop. A good recent description, however, on the integrated nitrogen policies to restore the habitat quality is given in Schoukens (2017). We also did not get any replies from Denmark and Switzerland in the first mailing contacts, although the latter country is performing a lot of research in the field (e.g. Rihm et al. 2016; Seitler et al. 2018) and recent research indicates clear positive of reduced N-deposition on the species diversity (Roth et al. 2013; 2019). Later, we were able to get into direct contact to Swiss researchers and finally on the Dessau workshop we received a very detailed presentation on the ammonia topic from Switzerland.

A very detailed initial reply came from an air quality researcher at Flanders Environment Agency (VMM). Ammonia is a big issue only in the northern part of the Belgium, while in the mountainous Wallonia the topic is not on the political agenda. Latter is mainly due to the lower livestock intensity in the southern provinces. In Flanders, ammonia emissions from new infrastructures are evaluated with regard to the expected nitrogen deposition in surrounding nature areas and Natura2000 sites. However, the assessment does not rely on critical levels but rests on the (empirical) critical loads for the resulting N deposition. The Flemish government is developing a so-called Programmatic Approach to Nitrogen (PAS) for the region similar to that one established in the Netherlands. Although the integrated PAS is not yet legally approved, the results are already used in environmental planning. In order to obtain a permit for a (new or altered) activity, nitrogen deposition is calculated using a dispersion model. VMM is not involved in the actual permit process itself, but plays an important role in generating the data used for this evaluation, as the organisation is responsible for (1) the emission inventory of air pollution sources in Flanders, including ammonia and nitrogen oxides, and (2) the dispersion and deposition modelling of nitrogen.

Ammonia emissions in Flanders are not based on actual measurements, but are calculated from activity data and emission factors. Assessments are made by ILVO (Research Institute of Agriculture, Fisheries and Food) with a focus on NH_3 and particulate matter near agricultural sources. For the emissions from other facilities, such as biogas plants, wastewater treatment plants, the responsibility for surveillance depends on the scale of the facility. Data are based on direct measurements and process-based statistics (PRTR activities). VMM and ILVO are jointly acting to improve the ammonia emission inventory. Because the reliability of the emissions impacts the entire decision-making process, calculation tools for agricultural emissions need to be updated from time to time. Activity data are provided by the department of agriculture but emission factors differ between countries e.g. due to differences in stable types and management practices. Belgium uses similar models than the Netherlands (OPS from RIVM) and these models have a resolution of 1*1 km. At the local level, the OPS output has recently been combined with a smaller-scale model.

VMM is planning, coordinating and carrying out the monitoring of ammonia in ambient air in Flanders. The organisation operates a long-term monitoring network for ammonia with passive samplers (currently 18 sites). The aim of this network is to assess the regional status and trends of ammonia concentrations (Table 3). Furthermore, VMM performs short term campaigns in nature areas (100 temporarily locations in 2015-2016) and ammonia measurements in the lee of stables. Additionally, two sites have been equipped with a miniDOAS system (in cooperation with RIVM), where highly resolved ammonia measurements can be taken.

Vegetation surveys in Flanders are generally within the responsibility of INBO (Research Institute for Nature and Forest) to establish a so-called "nature value map", but ammonia emitting facilities are not specifically dealt with in the program. Generally, N deposition is also a topic in the Belgian monitoring obligations within the international LTER and the ICP Forest Intensive Monitoring Programs.

The Belgian colleague does not know of new scientific findings or recent experimental studies on the effects of ammonia on plants and the vegetation. With regard to new technical equipment, he believes that highly resolved ammonia monitoring will not become available in the near future. and requires large investments and operational cost. However, the uncertainty in the measurements with diffusive samplers is low and various sampler types are available that can measure down to 0.5 µg m^{-3} and less. So, passive samplers combine a reasonable accuracy and precision with a low cost and easy use, and are therefore a suited device to check exceedances of critical levels (1 and 3 µg m^{-3} annually).

One feedback came from CEH Edinburgh (Scotland). The contacted person gave a bundled response to all the issues raised in our query. Rather than informing us about concrete research projects and administrative or legislative issues dealt within the UK, an extensive list of scientific references was provided in the mail. During the last 20 years, CEH Edinburgh has been involved in various international nitrogen initiatives (Hicks et al. 2011, 2014 a, b and Sutton et al. 2014) and continues to collate data from an integrated NECD Article 9 network on behalf of Defra. The network of impact monitoring sites will have to report every four years. In pioneering work, the institute developed the ALPHA® and DELTA® methods for ammonia measurements, which have the required sensitivity (limit of detection) to measure below the 1 and 3 µg m^{-3} (annual mean) critical levels (Tang et al. 2009; Puchalski et al. 2011; Tang et al. 2018; Martin et al. 2019). These methods are being applied successfully within the UK National Ammonia Monitoring Network (established in 1998) as well as in the Environmental Change Network (ECN) and Long-Term Monitoring Network (LTMN), where air quality impacts (including ammonia) and climate change are related to ecosystem change. CEH is also involved in the FRAME and EMEP4UK modelling projects running various scenarios for mitigation options (e.g. Flechard et al. 2011; Dore et al. 2015; Vieno et al. 2009, 2014; Bash et al. 2015 and Hellsten et al. 2018).

Research papers of CEH scientists dealing with the results of the Whim Bog Experiment will be presented later in the chapter, but research also covers other ammonia related areas, e.g. how management practice and planting schemes (buffer plantings) can reduce the adverse effects of ammonia (Bealey et al. 2014 a, b; 2016 and the reports of Dragosits et al. 2015 a, b).

Effects related ammonia research in the UK comes mainly from Scotland and is mainly focusing on bog and heath ecosystems, whereas almost no information is available from the other UK regions with high livestock intensities (e.g. East Anglia, N-Ireland and N-England). Recent reports (Hall et al. 2017; 2018; Rowe et al. 2019) have addressed the strong regional variation of ammonia levels and investigates which of the N-sensitive ecosystems are mostly exposed to ammonia. 60% of the UK land area currently receives ammonia concentrations above the critical level set to protect lichens and bryophytes (1 µg m^{-3}). This represents 85.3% of England, 56.3% of Wales and 17.8% of Scotland and 87.5% of Northern Ireland. However, only 3% of the UK land area receives ammonia concentrations above the critical level set to protect vascular plants (3 µg m^{-3}), ranging from 0.1% of Scotland to 17.2% of Northern Ireland. In Germany, these figures would certainly be much higher. Scientist of the CEH were also participating at the Dessau workshop and we would like to acknowledge the input of Matthew Jones (Centre for Ecology and Hydrology, CEH) who helped us to improve the literature review in the workshop proceedings, which is a short version of the following chapter 2.2. We also were able to get much informal input with regard to WP3, which deals with options to develop field-based approaches including field fumigation systems. The Dessau Workshop Proceedings have meanwhile been finalised (Franzaring & Kösler 2023) and give a concise summary of the meeting, including the talks that were given during the two-day gathering of over 100 participants.

2.2 Literature review

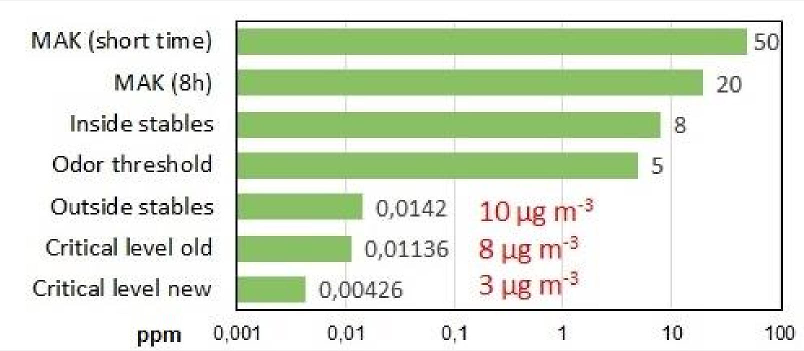
In order to evaluate the validity of current critical levels and to prepare our own fumigation study (WP2/WP3), we screened the literature on ammonia effects on vegetation from the last twelve years, focussing primarily on original publications which cited Cape et al. (2009a). Nevertheless, we also reviewed important publications prior to 2009 including fumigation studies from the

1970-1990s and studies that did not cite Cape et al. (2009a), that were helpful to better differentiate between phytotoxic vs. chronic effects and to address key points that needed to be respected in fumigation experiments. Besides the peer-reviewed literature, we also included various reports from studies that monitored ammonia in regional and national projects (see following subchapters.).

Air pollution from ammonia (NH_3) is relevant for human health and ecosystems. The gas is easily converted into NH_4^+, which can act as a plant nutrient, either by assimilation via the plant shoot (after entering the stomata) or after deposition to the ground and the subsequent uptake by the roots (Sutton et al. 1993; Fangmeier et al. 1994). Together with the oxidized forms of nitrogen, it is significantly contributing to the widespread eutrophication and acidification of ecosystems. As a result, the biodiversity of ecosystems is threatened at the expense of slow-growing nitrogen-sensitive, often rare and protected species (Bobbink et al. 2011, 2022).

Approximately 95 % of the ammonia (NH_3) emissions stem from agriculture, especially from livestock farming and the application of slurry or mineral fertilizers (UBA 2021). The odour threshold of ammonia is 5 ppm (3.8 mg m^{-3}) and the permissible workplace concentration (MAK) is 20 ppm (15 mg m^{-3}). Inside stables and after slurry application in the field, peak concentrations can be above 1 ppm (760 µg m^{-3}), whereas own measurements have shown that outdoors and close to stables, mean levels will still be around 50 µg m^{-3}.

Textbox: Ranges and reference limits of ammonia concentrations



Conversion at T=20°C

1 ppm = 0.71 mg m^{-3}

1 mg m^{-3} = 1.42 ppm

Source: Own illustration, University of Hohenheim.

Ammonia concentrations (per volume and mass, note logarithmic scale) that are relevant for health and the environment. "MAK "is the maximum permissible concentration at the workplace. Inside stables, concentrations are in the range from 5 to 8 ppm. Ammonia can be smelled by the human nose only at concentrations above 5 ppm (e.g. in the field after slurry application). Close to stables, concentrations will be >10 µg m^{-3}. The old and new critical levels are 8 and 3 µg m^{-3}, respectively.

Slightly elevated concentrations of the gas in the range of 10 µg m^{-3} can also be detected near waste-water treatment plants. Major roads can act as line sources, but concentrations drop away from the edge of the road (e.g. Cape et al. 2004; Bell et al. 2016; Elser et al. 2018). In remote areas, ammonia concentrations are often below 1 µg m^{-3} (UBA 2020), while the presence and excrements of animals (urea and uretic acid) and the degradation of plants will elevate levels locally. The text box above gives an overview of concentrations ranges of ammonia that can be expected in the environment.

2.2.1 Research in the early years

Experimental exposure of plants to ammonia in the 1970-2000s was mainly based on the use of pure (technical) NH_3 supplied from gas flasks to the air inlet of small closed fumigation chambers or continuously-stirred tank reactors (CSTR). Very high concentrations were applied initially to examine whether the growth of crop plants, e.g. sunflowers, could be stimulated in growth by nitrogen containing gases (Faller 1972; Hutchinson et al. 1972; Ewert 1979; Temple et al. 1979; Farquhar et al. 1980). In these short-term experiments with young plants, phytotoxic responses (foliar injury and growth reductions) were observed only at concentrations above 800 µg m^{-3}, depending on the plant species and the soil nitrogen supply. At concentrations below 4 µg m^{-3} fertilized crops and older plants were observed to not take up ammonia and release the gas during senescence (Cowling and Lockyer 1981). Unfortunately, many of the early and more recent studies did not mention sufficient details how the ammonia fumigation was controlled, whether constant levels were achieved and how the realized concentrations were determined. Optimally, the relationships between the inlet and the outlet NH_3 concentrations would have to be monitored constantly (Jones et al. 2007a). On the other hand, it will also be necessary to give a better account of how the test plants (receptors) were grown and what nutrient supply they received prior and during the fumigation. An overview of published ammonia fumigation experiments prior to 2009 as well as some more recent papers (going to be mentioned in chapters 2.2.2 – 2.2.4) is given in Table 1.

Dry deposition, bi-directional fluxes and compensation points of ammonia have been addressed early by Scottish, Dutch and Danish scientists (e.g. Sutton et al. 1993; Duyzer 1994 and Husted and Schjørring 1996) and have been parameterised in air-vegetation models later (e.g. Loubet et al. 2001; Nemitz et al. 2004). The fluxes are driven by changes to the equilibrium between the ammonia concentrations in the air and in the sub-stomatal cavity of plant leaves and depend on the air/leaf temperature, stomatal and cuticular resistances, seasonal and diurnal changes in stomatal opening, plant water status, ammonium (NH_4^+) concentration (nutrient status) and pH of the apoplastic solution. Farquhar et al. (1980) and van Hove et al. (1987) obtained compensation points of around 5 µg m^{-3} in crop plants, but suggest that in oligotrophic vegetation, e.g. peatlands, heath and dunes, the uptake of ammonia may occur at lower concentrations (e.g. Jones 2007b showed an average compensation point of >0.4 µg m^{-3} for moorlands at background atmospheric concentrations). This is principally confirming that low concentrations in the range of the suggested critical levels can be "sensed" by vegetation in pristine areas, but as mentioned above, ammonia concentrations in agricultural and densely populated regions will only rarely be that low. During the lifetime and with the biological activity of plants during seasons, fluxes of ammonia will change. While young N-demanding plants are often a sink of ammonia in spring, old senescing plants may be a source and release the gas in the autumn. In addition, ammonia can be transported from a highly fertilized crop canopy to an adjacent natural vegetation with a lower compensation point and vegetation on acidic soil substrates will probably be more prone to the deposition of ammonia. Following the initial discussion of critical levels for ammonia at the UNECE Workshop in Egham 1992 (Ashmore and Wilson 1994), European research networks had been established in the 1990s to monitor atmospheric processes that lead to the deposition of oxidized and reduced nitrogen species. It became clear that ammonia has a short life-time (Asman et al. 1998) reaching acute concentrations close to emission sources. Furthermore, chronic responses in sensitive species, especially in oligotrophic ecosystem, are driven by the joint deposition of various N-containing compounds. In order to account for the differences between short-term atmospheric concentrations and the cumulative annual inputs, the concept of critical loads had been introduced.

Ground-breaking research on the adverse effects and dose-response functions of ammonia on the natural (e.g. nitrogen-poor heathland) vegetation came from the Netherlands and served as the basis for the UNECE critical level of 8 µg m^{-3} (van der Eerden et al. 1982; Dueck 1990; van der Eerden et al. 1991; Dueck and Elderson 1992). Research of van der Eerden (1982) was based on the use of diluted exhaust from stables in closed or open top chambers (OTC), while later studies in Wageningen (Dueck and van der Eerden) involved technical ammonia supplied via mass flow controllers. Concentrations were in the range of 50 to 100 µg m^{-3} and were determined with NOx converters/monitors. While there were several OTC experiments in the UK addressing NH_3 and wet deposition of N in the 1990-2000s, the only comparable research project performed in Germany dealt with an ammonia fumigation of three grassland species grown in monocultures and mixtures. The experiments were performed in OTCs with diluted technical ammonia using set concentrations of 20 and 50 µg m^{-3} (Jäger et al. 1998). However, the realized concentrations could not be documented due to failures of the NO_x converters. Summaries of the pioneering research of ammonia effects on plants can be found in the reviews of Adaros and Dämmgen (1994), Fangmeier et al. (1994) and Krupa (2003).

Based on the above-mentioned studies, we suggest that ammonia concentrations were probably too high in the chamber-based experiments from 1970 to ca. 2000, restricting the validity of results with respect to the old and new critical levels. Expert judgements and extrapolations from high to low concentrations were used and long-term responses were predicted from short term experiments. None of the mentioned fumigation experiments operated in the range of existing environmental concentrations mainly due to technical reasons. When using pure ammonia without a time and concentration-controlled feedback system, peaks and accidental overdosing may occur that will lead to short-term burdens on plants eventually triggering acute effects. In fact, this will likely also happen in natural vegetation that is exposed to the fumes and plumes from adjacent slurry spreading or livestock housing.

Table 1: Overview of ammonia fumigation experiments in closed and open chambers

Source	Fumigation type	Supply of ammonia	Set range in µg m^{-3}	Method of ammonia measurement	Data on realized concentrations	Duration	Plant species
Faller (1972)	Glass chambers, 0.5 m^3	diluted NH_3, daylight hours	0 - 1600	none, calculated from flow	No	3 weeks	*Helianthus annuus*
Hutchinson et al. (1972)	Plexiglass chambers, 0.3 m high, 0.15 m sides	diluted NH_3,	24-44	trapped in KCl solutions	24-44	24 hrs	*Glycine max, Zea mays, Helianthus annuus*
Ewert (1979)	2 chambers of 10 m^3	diluted NH_3, 24 hrs day^{-1}	700 - 21000	air sampling, Nessler´s reagent	No	1-500 hrs	48 woody species, 10 crops
Farquhar et al. (1980)	Glass chamber, 0.2 m^3	diluted NH_3, 24 hrs day^{-1}	0 - 50 nbar	air sampling, indophenol method	No	20 minutes	*Phaseolus vulgaris* L. and other
Cowling & Lockyer (1981)	Exposure chambers	diluted NH_3, 16 hrs day^{-1}	0, 148 and 550	air sampling, Nessler´s reagent	No	40 days	*Lolium perenne* L.
van der Eerden (1982)	Open Top Chamber, closed chambers	Exhausts from poultry and pig manure, 24 hrs day^{-1}	600 - 4500	air sampling, Nessler´s reagent	No	3 - 60 days	grasses, trees, *Brassica*, *Lycopersicon*
Lockyer & Whitehead (1986)	Exposure chambers	diluted NH_3, 16 hrs day^{-1}	0, 100 and 500	air sampling, indophenol method	16, 118, 520	40 days	*Lolium multiflorum* Lam.
van Hove et al. (1987)	Leaf Chamber	diluted NH_3, short term	0 - 400	Chemiluminiscence after converting NH_3 to NO	No	some days	*Phaseolus vulgaris* L.
Whitehead & Lockyer (1987)	Exposure chambers	diluted NH_3	0 - 700	air sampling, indophenol method	14 - 709	33 days	*Lolium multiflorum* Lam.
Dueck (1990)	Open Top Chambers	diluted NH_3, 24 hrs day^{-1}	0, 53 and 105	Chemiluminiscence after converting NH_3 to NO	No	9 months	*Calluna vulgaris*
Dueck & Elderson (1992)	Open Top Chambers	diluted NH_3, 24 hrs day^{-1}	0, 50	Chemiluminiscence after converting NH_3 to NO	No	9 months	*Arnica montana, Viola canina, Agrostis capillaris*
Husted & Schjørring (1996)	Glass cuvette, 0.45 m^3	diluted NH_3, 24 hrs day^{-1}	0 - 25 nmol mol^{-1}	Chemiluminiscence after converting NH_3 to NO	No	twice a day, 7 days	*Brassica napus* L.
Jäger et al. (1998)	Open Top Chambers, 3.15 m diameter	diluted NH_3, 24 hrs day^{-1}	20 and 50	Chemiluminiscence after converting NH_3 to NO	No	3 years	Monocultures and mixtures of *Bromus erectus* Huds, *Brachypodium pinnatum* P. Beauv. and *Arrenatherum elatius* P. Beauv.
Geßler et al. (2002)	Borosilicate twig chambers	diluted NH_3, 24 hrs day^{-1}	100	continuous wet flow denuder AMANDA	No	several hours	*Picea abies*
Adrizal et al. (2006)	Exposure chambers	diluted NH_3, 24 hrs day^{-1}	2800 - 5000	Photoacoustic NH_3 detector	No	12 weeks	*Juniperus virginiana*, *Gleditsia triacanthos*, *Phalaris arundinacea*, *Populus* spec. *Salix* spec.

Source	Fumigation type	Supply of ammonia	Set range in µg m^{-3}	Method of ammonia measurement	Data on realized concentrations	Duration	Plant species
Jones et al. (2007a)	Flux chamber, 1.84 m^3, placed in an OTC at Bush Estate near Edinburgh, Scotland	1% aqueous NH_3 solution at different rates and concentrations	1-100	AMANDA system (Ammonia Measurement by annular Denuder sampling with on line Analyser system)	daily resolution	6 hours each trial over 3 to 6 weeks	mixed turfs made up of *Calluna vulgaris* (L.) Hull, *Eriophorum vaginatum* L., *Sphagnum capillifolium* (Ehrh.) Hedw. and *Sphagnum papillosum* Linb.
Jones et al. (2007b)	Flux chamber, 1.84 m^3, placed inside of an OTC	1% aqueous NH_3 solution at different rates and concentrations	1-140	continuous wet flow denuder AMANDA, measurements at inlet and outlet	daily resolution	6 hours each trial over 3 to 6 weeks, stomatal and non-stomatal species	*Deschampsia cespitosa* (L.) Beauv., *Calluna vulgaris* (L.) Hull, *Eriophorum vaginatum* L., *Cladonia* spp., *Sphagnum* spp., and *Pleurozium schreberi* (Brid.)
Jones et al. (2008)	Flux chamber, 1.84 m^3	release of ^{15}N labelled NH_3 from $(^{15}NH_4)_2SO_4$ with NaOH	16 - 20	continuous wet flow denuder AMANDA	daily resolution	15 days	*Calluna vulgaris*
Franzaring et al. (2012)	Mini greenhouses	diluted NH_3, 24 hrs day^{-1}	0 - 200	Passive samplers	2.2 -195	88 days	*Brassica napus* L.
Ilogu Chibuzo (2012)	Exposure chambers	controlled evaporation of NH_4Cl solutions	0 - 84	Passive samplers	3, 25, 84	30 days	*Echinochloa crus-gallii* and *Lolium multiflorum*
Jones et al. (2013), presenting results from PhD Thesis of Jonathon Foot from 1998	Open Top Chambers	uncontrolled evaporation of NH_4OH solutions containing 0-10 % of ammonia	0 - 35	Passive samplers	Yes	28 weeks	Mixtures of *Dactylis glomerata, Plantago lanceolata, Festuca rubra, Centaurea nigra* etc.

2.2.2 Overview of the research published after 2009

It must be highlighted that during the last 30 years many research activities on the fluxes, deposition and effects of ammonia came from Scotland, namely David Fowler, Neil Cape and Mark Sutton, all scientists from the Centre for Ecology & Hydrology (CEH) Edinburgh. These authors have co-authored many studies and worked as PI´s of several EU projects that dealt with the deposition of acidifying and eutrophying gases and particulates. We only cited a few of these studies in previous chapter, but acknowledge the impact CEH scientists still have on current research.

In present sub-chapter we will take a closer look at research that deals with the effects on plants and the vegetation under more natural conditions. Whereas chamber-based experiments (see. 2.2.1) dealing with plant exposure to ammonia were mostly applying higher concentrations, more recent projects tried to investigate low-range ammonia effects in order to address potential responses in sensitive plant species and to be more representative for the range of critical levels.

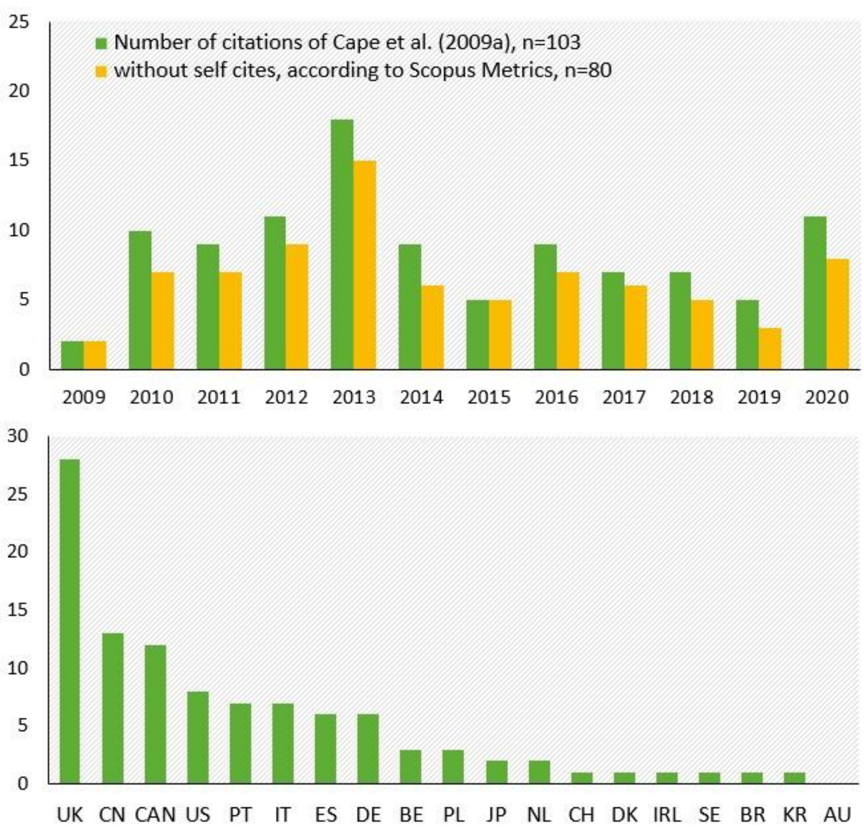
A screening of newer literature dealing with the recommended CLE and contributing new data from the UK and other European countries will be presented here. In Annex 1 (p. 159), we present an overview of 103 peer-reviewed references (as of December 2020 and updated to 122 in July 2023) that cited Cape et al. (2009a) according to the databank Scopus®. The bibliographic information of the retrieved papers as well as their abstracts, keywords and digital object identifiers (DOI) were collected and worked on using the Citavi 6.3 software, which is a powerful program to manage scientific information. A project database with the name of "NH3-critical levels" was created and the references were imported. All references that were cited in the seven unpublished interim reports, the background document and present final report have been appended to the database.

Figure 2 (top) gives an overview on how many citations per year were recorded and how many of these did not stem from the Scottish research group that also authored the Cape et al. publication. After a decline in the numbers of citations until 2019, the topic "critical levels" peaked again recently and we can observe that 80 % of the citations were from groups not involved in writing the cited paper.

When looking at the country of residence of the main author and affiliation (Figure 2 below), we can see that most publications which cited Cape at al. (2009) stemmed from the UK, China, Canada and the US, being followed by three Mediterranean countries mostly studying lichen diversity among ammonia gradients. Interestingly, in the Netherlands, Denmark and Germany, the research on effects of ammonia is not of major importance, although the countries have a very large livestock production sector and severe problems with eutrophication. It must be noted that until the late 1990s, most of the ground-breaking research ammonia impacts had been performed in the Netherlands (the research group of Dr. Ludger van der Eerden at Plant Research International in Wageningen). The old CLE of 8 µg m^{-3} had originally been derived from fumigation studies from that institute and in those years, there were many ammonia-related research programs funded by the Dutch, UK and German governments. An even stronger absence of research on ammonia was noted in France, a country with a strong livestock sector in the EU, where no peer-reviewed literature on the effects of ammonia was published after 2009.

In order to get a first impression on the topics dealt with in the papers, a list of 64 keywords was created that were assigned to the articles after reading the abstracts and downloading many of the publications. Since the university of the contractor did not have licenses for all of the journals, some papers could not be retrieved. If we found a paper highly relevant to the topic of critical levels, we asked the corresponding author to send us a personal copy of the article.

Figure 2: Citations of Cape et al. (2009a) in the peer-reviewed literature, broken down after years (top) and countries of residence of the main author (below)



Source: Own illustration, University of Hohenheim.

When looking at the specific topics dealt with in the 103 articles, the study of the presumed ammonia sensitivity of lichens plays an overwhelming role. 27 articles focus on the mapping of nitrogen sensitive lichens with many published from Southern European countries, despite the limited intensive livestock. Another quarter of the citing articles dealt with the modelling of N deposition and 20 articles were presenting data from ammonia measurements using passive samplers. 16 studies investigated changes in the species composition. 11 studies reported about "bioindication" methods, addressing ecophysiological changes in lichen and bryophyte species and vascular plants along ammonia gradients. Only a few of these studies used pre-cultivated vascular plants, which were actively exposed in the field at locations, where ammonia was measured. In the recent paper of Ellis et al. (2022) on potential nitrogen risks to Himalayan forests, the results of 14 lichen biomonitoring studies were used to derive independent estimates for three nitrogen pollutant types. These were the critical level for ammonia concentration (µg m^{-3}), the critical load for total nitrogen deposition (kg N ha^{-1} yr^{-1}) and the total throughfall nitrogen deposition. Based on their calculations for diverse responses on lichens (e.g. total species richness, sensitive species richness, 'forage lichen' abundance and cyanolichen abundance), the critical level for ammonia would be 1.44 µg m^{-3} as a grand mean and the critical load (wet and dry, reduced and oxidized) would average to 8.26 kg N ha^{-1} yr^{-1}.

The results we found imply that mosses might generally stand higher concentrations of ammonia than lichens, but it must be noted that there is not enough information for mosses in the moment (Armitage et al., 2014). We must also point to the fact that there is a vast ecological amplitude among the 1100 moss species in Germany that complicates generalizations. The responses to ammonia will be quite different between acidophytes and basiphilous species and will be variable between bryophytes, liverworts and hornworts.

With regard to lichens, we need to be aware that their potentially higher sensitivity to air pollutants relates to the fact that these organisms are actually made up of at least two species (see text box below). The functioning of the community “lichen” is very sensitive to environmental changes, but it is not always clear, which single factor disturbs the well-balanced interactions between the two or three species.

Text Box: On the biology of lichens and potential reasons for their sensitivity to ammonia

- Lichens are composite organisms consisting of algae (or cyanobacteria) and fungi. The algae perform photosynthesis to generate energy and sugars, while the fungus lives from the photo-assimilates and in its hyphae (filaments) creates a specific microenvironment and microclimate that protect the algae. The two species live in a so-called mutualistic relationship. However, disentangling their relative contribution is challenging and a comprehensive trait–environment framework addressing such questions is missing in lichens (Hurtado et al. 2020).
- Lichens are non-parasitic, do not have roots but attach to and grow on substrates (i.e. rocks and tree barks), which must have a specific pH and roughness. Lichens are associated with typical host trees and grow only in the mild and moist season.
- They receive nutrients from dust, the rain water and the water films that solve nutrients from the substrate they are growing on. In dry seasons growth of lichens ceases because there are no mechanisms (e.g. cuticles) to protect the organism from desiccation.
- They are poikilohydric, i.e. they have no mechanisms to actively balance their water status.
- They are long recognised bioindicators (urban lichen deserts) for air pollution and nitrophytic and nitrophobic lichens have been identified.
- Their potential use for the bioindication of ammonia has been suggested by e.g. Franzen-Reuter (2013) and Windisch et al. (2016), but according to Jovan et al. (2012), Mayer et al. (2013) and Manninen (2018), eutrophic lichen species do not particularly respond to NH_3 but also to oxidized forms of N.
- A lichen will increase in diameter between 0.1 to 0.8 cm per year only depending on the climate and the nutrient supply. It can be expected that nitrogen containing pollutants will speed up the growth of the algae, from which the fungus can profit as well. If the fungus and its hyphae however, do not grow fast enough, the algae might not be protected from harsh conditions anymore, so that the whole lichen will eventually die. It will be difficult to find a “point of no return” or a critical concentration of ammonia that defines when an initially positive effect of nutrition e.g. due to low doses of ammonia turns into an adverse effect that will eventually lead to the die-off of the organism and/or the disappearance of a lichen from the environment.
- According to van Herk (2022) a “major reduction of the world-wide ammonia emission is probably needed to save the many species of naturally acid bark. However, it is not possible to assess how great this reduction must be: deacidification of tree bark will continue anyhow, this process can only be slowed down. Therefore, there is no sustainable level: effects on acidophytes are cumulative (and susceptibility also strongly differing per lichen species).”

The fact that about 300 of the 2000 lichen species are threatened from extinction e.g. in Germany shows that these organisms are very sensitive to environmental change (Wirth et al., 2013). With regard to ammonia, the N-tolerant *Xanthoria parietina* and the N-sensitive *Evernia prunastri* are thought to be good bioindicators which can be used in passive and active studies in various countries. An epidemiological study in Switzerland (Urech et al. 2015) showed that the probability of nitrophobic lichens to occur in the ephiphytic vegetation will strongly decrease below an ammonia concentration of 3.3 µg m^{-3}. This is somewhat above the critical levels suggested by Cape et al. (2009a) for N-sensitive lichen and bryophyte species, but confirms that adverse effects of ammonia on the biodiversity indeed occur at low concentrations. The negative relationship was most pronounced for *Hypogymnia physodes* and *Pseudevernia furfuracea*.

Besides the agricultural sources, the NO_x emissions from industry, traffic and wastewater treatment sectors are also responsible for the losses in biodiversity of certain cryptogams and lichens. Sutton et al. (2020) have recently suggested that the successful reduction of SO_2 pollution in Europe and elsewhere has led to a general alkalinisation of rain and air that will be harmful to naturally acidophytic species. On the other hand, in the 1990s and early 2000s the elimination of acidification often favoured the return of rare basiophytic lichens. The impacts of the recent and ongoing changes in air quality have also been investigated in Sweden and showed a very slow recolonization of sensitive species due to existing dispersal limits (Weldon and Grandin 2021).

Only in the 1990s, research on the phytotoxicity of air pollutants moved to field-based fumigation systems to study the effects of ammonia on pre-cultivated plants or established natural vegetation. Such systems should operate under the normal ambient climates and should not create concentration peaks. Examples are the FACE (free air carbon dioxide enrichment) experiments and the use of open top chambers (OTC) e.g. to study adverse effects of ozone.

The only long-term field ammonia fumigation system is at the Scottish Whim Bog Field Facility (Southwest of Edinburgh) and has been in operation since 2002. The site simulates a point source release (e.g. chicken farm) producing a concentration gradient across an ombrotrophic bog, ranging from an annual average of around 80 µg m^{-3} (at 6 m) to ambient background concentration (<1 µg m^{-3}). The site represents an emission produced of approximately 5.000 chickens in a clean shed system (SCAIL model estimate) and is similar to annual concentrations reported near comparable farms (e.g. Pitcairn 1998). All major species have declined (including *Cladonia, Sphagnum, Calluna, Hypnum*) except the now dominant sedge *Eriophorum vaginatum*, across the large majority of the concentration ranges (van Dijk et al. 2017; Levy et al. 2019). Evidence suggests long term effects with impacts responding to changes occurring over several years (Sutton et al. 2020). Severe impacts of lichen bleaching recorded near the source may be caused by peak toxicity events, but impacts along the transect are likely caused by other mechanisms such as algal/fungal imbalances.

More recently in 2021, CEH has established a new field ammonia fumigation system at Glencorse Field Facility (Southwest of Edinburgh). This site releases ammonia at a similar level to Whim, but assesses the impact of ammonia within a woodland environment (pers. comm. Matthew Jones). The site has 20 m long fumigation pipes at three heights to ensure ammonia is relatively well mixed in the trunk region of the woodland. Ammonia is only released when wind directions and speed are adequate to ensure maximum concentrations are representative of concentrations expected downwind of intensive agriculture. Significant pre-testing of the system was conducted using a Picarro gas concentration NH_3 analyser in a multi-height inlet system, multi-height profiles of ALPHA samplers, a transect of ALPHAs and ALPHAs placed throughout the woodland to allow the researchers to establish the plume shape and concentrations under different meteorological conditions. The site also has a 15 m high meteorological mast to establish wind, temperature, relative humidity and leaf wetness profiles. The site is being used as a test bed to improve model estimates of NH_3 deposition to woodlands, NH_3 impacts on forest ecosystems, NH_3 impacts on GHG emissions, and the work will include assessment of NH_3 impacts on lichen transplants in relation to critical levels. A third site has also been established in March 2022 in Sri Lanka following the same intensive pre-measurements as at the Glencorse experiment, which will for the first time assess nitrogen impacts in South Asian ecosystems, and will be compared to a large-scale assessment of N impacts in the Himalayan Forests in South Asia.

A new generation of experiments mimicking the dry gaseous deposition of ammonia in established ecosystems is currently on the way also in China (Pan et al. 2020) and will be recognized under the name Free-Air NH3 Enrichment (FANE).

2.2.3 Gradient studies

While until the year 2009, several papers on adverse ecological effects of N-deposition in the vicinity of livestock husbandry and motorways had been published, only six papers citing Cape et al. (2009) were reporting about investigations on plants in the lee of ammonia emitters. These papers are Paoli et al. (2010, 2014), Staelens et al. (2012), Wuytack et al. (2013), Watmough et al. (2014) and Izquieta-Rojano et al. (2018). However, we found a few more interesting studies that did not cite Cape et al. (2009a) but which addressed nitrogen deposition gradients (Table 2).

In the publications on “gradient studies”, the observed effects are frequently plotted against the mean ammonia concentrations, which are decreasing with the distance from the source, most of the times livestock stables. Effects, i.e. ecotoxicological endpoints like nutrient concentrations, N:P ratios, chlorophyll concentrations or certain amino acids are assumed to stem only from the gas at question. Often such studies neglect the potentially confounding factors that are superimposing the results. These can be other phytotoxic pollutants (like NO_x), changes in the microclimate and wind patterns along the gradient and changes in soil conditions, if ground vegetation is investigated or when passive biomonitoring is applied.

Apart from the six mentioned papers, we were able to trace further publications, which will be mentioned in the present subchapter. We thought it necessary to differentiate between passive and active approaches to study plant responses along ammonia gradients and therefore created Table 2 with summaries of the main findings. After retrieving information on where the studies were performed, which emission sources and how many locations had been selected, we also compare the concentration ranges and how ammonia concentrations were measured in the different studies.

In the investigations, in which established plants had been used on site, i.e. the **passive approaches**, two papers dealt with lichens and one with a moss, *Hypnum cupressiforme*. Two studies focused on vascular plant species, namely heather in the Whim Bog experiment and oak leaves in the Belgian study (Wuytack et al. 2013). While the lichen studies (Paoli et al. 2010, 2014) found the critical levels to be 1.7 and 1.9 µg m^{-3}, the no observed effects concentration (NOEC) for the moss species was 3.5 µg m^{-3} in the Spanish study (Izquieta-Rojano et al. 2018). Latter value is well above the critical level suggested for lichens and mosses and is already in the range of the critical value that had been suggested for vascular plants.

In the **active biomonitoring** studies citing Cape et al. (2009), lichens also play a predominant role (Table 2 continued). Chlorophyll fluorescence is used as a promising response indicator since growth and vitality changes are difficult to determine and would afford longer time until significant changes can be detected. It must be noted that methods for supplying and cultivating transplants are not well documented. Since in the present project (WP2) we intended to expose lichens to chronic concentrations of ammonia over two years, we approached experts who might have some experience in cultivating lichens. Contacts to scientist from Germany, Switzerland and Italy had thus been established, but we could not get hold of a standard cultivation scheme. As mentioned later (see chapter 3.1.1) our lichen transplant/cultivation approach failed. The three mentioned lichen transplant studies had been performed in the lee of stables. While ammonia concentrations were well documented, information on microclimatic and light conditions along the gradients was not documented. In order to support the survival of lichens in field studies over longer times, the substrates (bark and twigs) will have to be kept moist from time to time to make sure that lichens have optimal growth conditions.

Based on the findings with lichen transplants, the validity of low critical levels for ammonia can be confirmed for lichen and bryophyte species. However, it remains unclear whether the response indicator chlorophyll fluorescence i.e. adverse effects on the photosynthetic energy conversion, has an ecological importance that can be related to the long-term survival of plants. Other ammonia gradient studies made use of vascular plants. Out of these, grasses play a predominant role as bioindicators since their upright growth habit and the presence of numerous stalks enable the effective scavenging and accumulation of air pollutants. This is the main reason why standardized grass cultures are being used to determine the effect of air pollutants on vegetation. While Leith et al. (2009) used grasses in the lee of Scottish poultry farms, Ilogu Chibuzo (2012) within a dissertation at the University of Hohenheim used grasses and N-demanding weeds in the lee of a Swabian pig stable. In both studies plant biomass increments and nitrogen concentrations proved to be sensitive indicators for the fertilizing influence of ammonia. We suggest that these responses are more meaningful ecological indicators or ecotoxicological endpoints than e.g. chlorophyll fluorescence since they directly relate to the mechanisms driving plant competition and vegetation changes in plant communities.

It may be expected that fast-growing species will be able to increase their growth more strongly due the input of gaseous and particle-bound nitrogen while the slow growing species will lose competitive ability and eventually die out. Numerous studies have proved that grasses have strongly invaded into European ecosystems subjected to long-term nitrogen deposition replacing small herbs, shrubs and mosses. Grass encroachment is generally recognized unfavourable for nature protection and enhancing the mowing frequency, i.e. the reduction of nitrogen available in the ecosystem, will lead to extra costs.

An early biomonitoring study to detect the uptake of ammonia under field conditions was performed by Sommer and Jensen (1991) in Denmark who used ryegrass and isotopic analyses in the lee of stables. The ^{15}N dilution method was also implemented in Germany by Mehlert et al. (1995) and Böhme et al. (2002) in the ITNI soil-plant system (Integrated Total Nitrogen Input) to check whether the N deposition at different sites agreed with the modelled rates. A similar approach using biomonitors was later presented by Hurkuck et al. (2015) using *Lolium multiflorum* and *Eriophorum vaginatum.*

Biomonitoring experiments were also reported by Leith et al. (2005, 2009) using *Deschampsia flexuosa* and *Lolium perenne* and also Ilogu Chibuzo (2012) used grass species to determine the effects of ammonia in the lee of large stables. Significant growth stimulations were only observed in a few of the sown grass species when concentrations were above levels > 10 µg m^{-3}. In order to produce enough biomass, biomonitoring studies should last for several weeks and the nutrient supply should be high enough to initially support the growth of plants but low enough to later force the plants to supply their needs from the airborne ammonium. Grasses are very suited biomonitors for airborne pollutants (VDI 2020) due to their large leaf canopy area, but critical levels for ammonia should also be tested for other plant taxonomic groups indicative for the rare and nitrogen sensitive species. Recently, Mohr and Suda (2017) presented a biomonitoring system (see Table 2) that makes use of evergreen mature *Calluna vulgaris* plants. The material stemmed from clones that had been cultivated ten months before the onset of the study and it was grown in inert quartz sand that was supplied with a nutrient solution.

One of the grasses that has expanded in many European heath and moorland ecosystems in recent decades is the Purple Moor Grass, *Molinia caerulea* (L.) Moench. Various studies were able to show that N-deposition was the main driver for its increased frequency (Aerts and Caluwe 1989; Berendse et al. 1994, Hogg et al. 1995; Damgaard et al. 2017). The grass species could thus be a suited active bioindicator to determine the deposition of N containing pollutants.

Using pre-grown clones in active biomonitoring instead of sown plants offers the advantage that variation between replicates will be low and that plant numbers per pot can be fixed. In transplants, more initial biomass will be available and the fresh mass of the ramets prior to and after the experiment can exactly be determined. In own experiments (Franzaring and Fangmeier 2006), we used clones of *Molina caerulea* L. Moench grown in quartz sand and found that plants grown closer to stables indeed produced more biomass. Shoot growth was positively related to ammonia concentrations (R^2 0.84), but since we used sand as an unfertilized substrate, the transplanted ramets did not produce much biomass during the three months of exposure (less than 3 g dry mass).

Table 2: Ammonia gradient studies based on passive and active approaches

Passive	**Izquieta-Rojano et al. (2018)**	**Watmough et al. (2014)**	**Wuytack et al. (2013)**	**Pinho et al. (2012)**	**Sheppard et al. (2011)**	**Leith et al. (2009)**
Country	ES	CAN	BE	PT	UK	UK
Emission source	Pig stable, 3500 pigs	Motorway densitiy in a region	Various stables	Barn with 200 beef cattle	Technical ammonia, Whim Bog experiment	Scottish poultry farms, 100,000 and 120,000 birds
Distances, number of points	7 points in an oak forest	17 forest plots	34 sampling points	74 lichen and 21 ammonia sampling points in cork-oak woodland	continuous 0 to 120 m from source	4 and 6 positions along transects of 250 and 276 m
Ammonia concentration range	1.5 - 48.8 µg m^{-3}	0.2 - 2.8 µg m^{-3}	1.9 - 29.9 µg m^{-3}	1.4 - 34 µg m^{-3}	not mentioned, converted to N-deposition using site-specific factors, <10 - >100 kg N ha^{-1} a^{-1}.	0.58 - 69.6 µg m^{-3} and 2 - 101 µg m^{-3}
Pollutants/ Equipment	ALPHA passive samplers	NO_2 and NH_3, self-built passive samplers	Radiello® passive samplers	ALPHA passive samplers	Denuders and passive samplers	ALPHA passive samplers
Methods and parameters	Ecophysiological analyses, N and protein contents, ^{15}N signatures	Vegetation relevées, N concentrations	specific leaf area, stomatal resistance and chlorophyll content	Species distribution, functional groups	species cover, plant damage (bleaching), litter accumulation over 7 years	Biomass, tissue N and ammonium
Plant species investigated	Moss *Hypnum cupressiforme*	Lichens, higher plants	Leaves of *Quercus robur* L.	Oligotrophic and nitrophytic lichen species	*Calluna vulgaris* L.	*Lolium perenne* L. and *Deschampsia flexuosa* L., no fertilizers
Conclusions on critical level and main findings	Change in tissue nitrogen was the best predicted response to ammonia. A critical level of 3.5 µg m^{-3} was derived using a site-specific NOEC. Would have been lower if ammonia background was lower.	1.7 µg m^{-3} for NH_3 and 30 µg m^{-3} for NO_2	Leaf morphology, chlorophyll content and ecophysiology are unaffected by ammonia. Results are confounded by edaphic and genetic factors.	Critical level is below 1.9 µg m^{-3}.	Dry gaseous deposition has greater effect than wet deposition, plants experience more drought and pathogen stress.	Biomass and tissue N respond along the gradient. Results confirm the critical level for N-sensitive higher plants of 2-3 µg m^{-3}. Biomonitoring is possible.

Active	**Paoli et al. (2010)**	**Paoli et al. (2014)**	**Munzi et al. (2014)**	**Ilugo Chibuzo (2012)**	**Laffrey et al. (2010)**	**Mohr & Suda (2017)**
Country	GR	IT	UK/PT	DE	FR	DE
Emission source	Sheep stable	Composting plant	Technical ammonia, Whim Bog Experiment and cattle barn in PT	Pig stables	Busy roads in Rhone-Alpes	Stables, remote areas
Distances, number of points	3 distances at 0, 60 and 5000 m	3 distances at 0, 200 and 400 m	3 distances at 12, 30 and 60 m	3 distances at 67, 149 and 804 m	Several distances from the road	6 sites, different distance to stables
Ammonia concentration range	1.3 - 62.4 µg m^{-3}	2.7 - 48.7 µg m^{-3} and unrecognized peaks with much higher concentrations	3 - 9 µg m^{-3} in the lee of the barn, no data for Whim Bog	1 - 33 µg m^{-3}	3 - 37 ppb NO_2, no ammonia measured	1 - 4 µg m^{-3}
Pollutants/ Equipment	Radiello® passive samplers	Radiello® passive samplers	ALPHA passive samplers	Radiello® passive samplers	Gradko passive samplers for NO_2	FERM sampler, TONIS bioindicator
Methods and parameters	Chlorophyll fluorescence after 30 days	Chlorophyll fluorescence and lichen diversity over the years	Chlorophyll fluorescence	Biomass, foliar N, amino acids	Biomass ^{15}N, foliar N	Biomasss, N contents in plants, soil and nutrient solution
Plant species investigated	Lichen transplants of *Evernia prunastri* and *Pseudevernia furfuracea*	Lichen transplants of *Xanthoria parietina* and *Evernia prunastri*	Lichen transplants of *Evernia prunastri* and *Xanthoria parietina*	*Lolium perenne* L., *Echinochloa crusgalli* (L.) P. Beauv., *Chenopodium album* L. and *Urtica dioica* L.	*Molinia caerulea* (L.) Moench	*Calluna vulgaris* (L.) Hull
Conclusions on critical level and main findings	Electron transport is a sensitive indicator. In the future, minimum concentrations and exposure times need to be tested to derive critical levels for the Mediterranean region.	Replacement of the acidophilic lichen flora by nitrophilic species is likely to happen in the future. Lowest concentration is already above the critical level.	Oligotrophic species are better suited than nitrophytic species for the establishment of critical levels. NH_3 threshold for *E. prunastri* is below 3 µg m^{-3} since there were hardly any occurrences above this concentration.	Biomass, tissue N and amino acids respond to the higher ammonia concentrations in 3 out of 4 species.	Stronger biomass increment closer to the street, ^{15}N signature typical for diesel exhaust, higher foliar N levels. The grass is a suited bioindicator.	The system allows for a full nitrogen budget in actively exposed bioindicator plants as it derives the nitrogen contents in each of the compartments, including the water supply system and the soil.

M. caerulea was also used by Angold (1997) and Laffrey et al. (2010), but here the grass was used as a nitrogen biomonitor in the vicinity of roads to determine the effects of NO_x. While Angold (1997) transplanted the ramets into the native soil at fixed intervals near a dual carriageway near Birmingham, Laffrey et al. (2010) planted 27 seedlings into pots that were exposed at different distances to a motorway in Savoy. The British study only reported total leaf length as a growth parameter, while the maximum dry mass in the French study was reported to be 1.3 g. Indeed, Laffray et al. (2010) were able to show that the grass increases in growth due to the input of NO_x and NH_3 along French roads. The method they implied was originally developed at the Institute of Landscape and Plant Ecology of the University of Hohenheim (the contractor), but in the study of Kopsch (2011) we could not observe a stronger growth or higher N contents in pre-cultured and weighted plants at elevated concentrations of ammonia. In order to clarify whether the grass is stimulated by ammonia, we included pre-cultivated specimens of the species in our fumigation experiments (WP2). Other studies using grasses to indicate a growth stimulation were Cowling and Lockyer (1981) and Sommer (1988) based on *Lolium multiflorum* and Hurkuck et al. (2015) using the ^{15}N dilution technique in both ryegrass and *Eriophorum vaginatum*. Also, Mohr and Suda (2017) were aiming at the development of nitrogen bioindicators, but used *Calluna* instead of grasses. We will come back to the development of bioindicators for ammonia in WP3 and will come up with further literature on that topic in chapter 4.2.

2.2.4 Fumigation studies after 2009

Apart from the free-air exposure at Whim Bog, which uses the release of technical ammonia along a wind gradient, only two peer-reviewed papers on ammonia fumigation could be found that had been published after 2009. In our own experiment (Franzaring et al. 2012), we studied the effects of sub-acute concentrations of ammonia on the re-growth of winter oilseed rape. We could show that the crop is well able to benefit from the gaseous fertilizer at a concentration of 195 $\mu g\ m^{-3}$, but we did not test whether slightly raised or low concentrations of ammonia would be able to significantly interact with the physiology of the species. The second study on ammonia fumigation was published by Jones et al. (2013) based on original research from the late 1990s. The authors investigated the effects of ammonia deposition to plants from Welsh sand dune ecosystems and exposed mesocosms of plant mixtures in OTCs. Shoot biomass and tissue nitrogen increased significantly with increasing concentrations of ammonia. A significant biomass increase of the plant mixture was already observed when increasing the ammonia concentrations from 0.4 to 4.3 $\mu g\ m^{-3}$, i.e. dose equivalents of 2 and 24 kg N $ha^{-1}\ y^{-1}$.

2.2.5 Ammonia monitoring

Since the 1990s, pioneering work on ammonia measurements in the ambient air has been done in the Dutch Landelijk Meetnet Lucht (LML) and in the UK National Ammonia Monitoring Network (NAMN). Various types of denuders and passive sampler devices are being applied to allow for a resolution ranging from days to weeks.

Only recently, other devices have been made available for fast, continuous, real-time monitoring of ammonia. Examples are devices based on differential optical absorption Spectroscopy (DOAS). While the methodology is well able to determine low background concentrations, only a few sites have been equipped with these rather expensive and support demanding devices in the Netherlands and in Switzerland (Volten et al. 2012a; Volten et al. 2012b; Berkhout et al. 2017; Bell et al. 2017). Pogány et al. (2016) and Niederhauser (2017) therefore formulated the need for more intensive metrological research focusing on the quality assurance, inter-comparability and validation of ammonia in the ppb range. Meanwhile, new generation ammonia gas analysers, e.g. based on Cavity Ring-Down Spectroscopy have been placed on the market that may be included in research and monitoring initiatives in the near future. A very comprehensive recent overview of measurement techniques has been presented open source by Twigg et al. (2022) and will be discussed by the research community.

A first international initiative comparing different active and passive analysers was set up within the European MetNH3 (Metrology for ammonia in ambient air) project. One of the work packages of the MetNH3-Project dealt with the testing and calibrating of ammonia passive samplers. Samplers were tested in the UK's National Physical Laboratory's (NPL) controlled atmosphere test facility (CATFAC) and in the field at the above-mentioned Scottish Whim Bog Experimental site. The inter-comparisons between different sampler types have been introduced by Braban et al. (2018) and Martin et al. (2019). Measurement errors of the tested passive samplers varied between 11 and 23 % at the reference concentration of 1 µg m^{-3}, i.e. a concentration representative for the lower critical level. ALPHA passive samplers proved to be the best suited for the lower concentration ranges and have an exposure time of 28 days, whereas e.g. the Radiello® samplers can be used to investigate shorter periods.

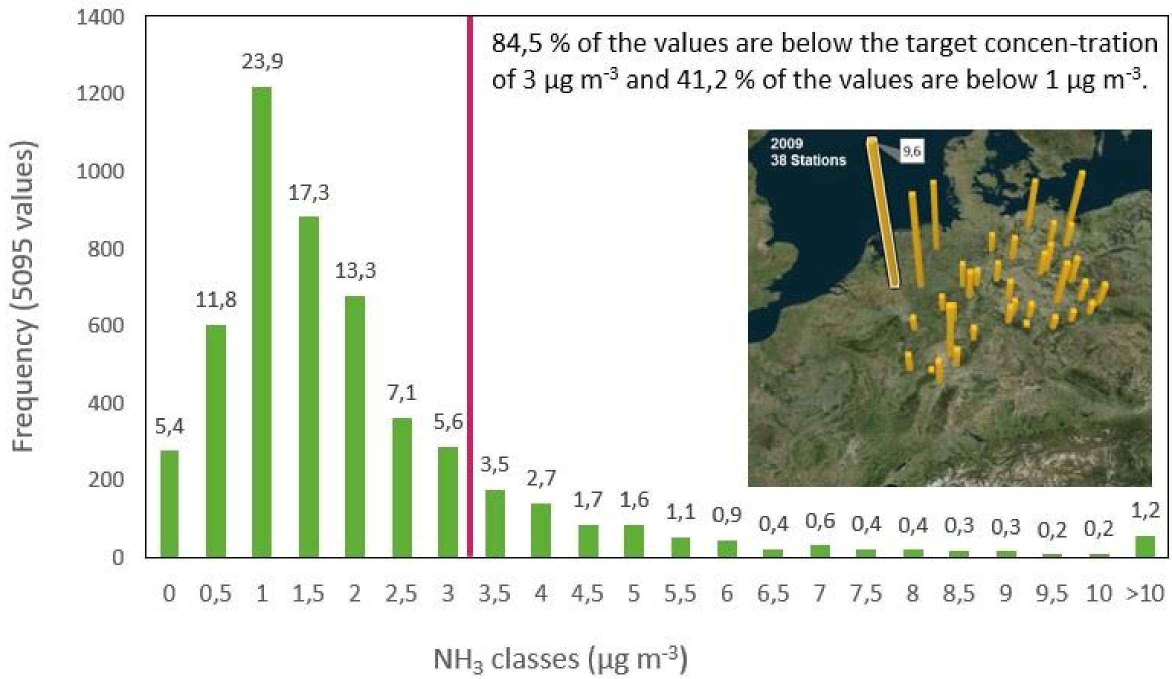
Here we focus on the results determined by passive samplers. These cost-effective devices have been developed for the determination of gaseous air pollutants in locations without power supply and are the only method to monitor gas concentrations in remote areas. In order to assure their quality, devices need to be certified according to CEN (European Committee for Standardization) standards EN 13528-1 to 3 for diffusive samplers. Two types are available, axial (badge type) and radial (tube like) samplers. Latter types have higher sampling rates and can therefore be exposed over shorter times (Puchalski et al. 2011).

Table 3 gives an overview of investigations that used passive samplers to study ammonia concentrations in different countries. Only papers and grey literature are listed that were published after 2009. In most of the studies, ammonia concentrations did not decrease over time contradicting the results from modelled concentrations (Wichink Kruit et al. 2017) and the information derived from emission inventories. As previously mentioned, the reasons for higher levels may be the reduced concentrations of SO_2 and increased volatilization of the gas due to the higher ambient temperatures. At the same time, higher concentrations may also stem from previously underestimated emissions, e.g. due to uncertainties in emission factors or activity data in agriculture, from waste water treatment facilities and biogas plants as well as from the increased use of diesel exhaust fluids (DEF, AdBlue). Higher vehicular emissions of NH_3 and ammonia slip have been addressed by Elser et al. (2018), Suarez-Bertoa and Astorga (2016) and Fenn et al. (2018).

In contrast to the pioneering European countries the Netherlands (Lolkema et al. 2015; Noordijk et al. 2020), Belgium (VMM 2017), Switzerland (Thöni et al. 2018) and the UK (Tang et al. 2018), countries with intensive agricultural systems like France, Italy and Germany do not operate national ammonia networks yet, so that regional data are scarce in these countries. We could only find one publication from France that reports on ammonia measurements in five agricultural and six forest regions (Fauvel et al. 2019). In Brittany, a region with intensive livestock farming, ammonia concentrations are on average in the range of 9 µg m^{-3}, while inside forest areas in the south and in the east, concentrations of less than 1 µg m^{-3} are common. Belgium, the Netherlands and Ireland have established monitoring sites in a large number of Natura2000 (the EU Habitat Directive) areas (Table 3). Since protected landscapes are often nitrogen limited, the vegetation (e.g. bogs and heathlands) is an endangered receptor for potentially harmful ammonia. In Germany, there have been some efforts in a few federal states to monitor ammonia with passive samplers, mainly badge type devices (e.g. Lohrengel et al. 2012; LfU 2019). In Germany, no continuous national ammonia monitoring network exist and the activities are restricted to partly temporary Federal State initiatives (Table 3), Based on the supply of data from the Thünen Institute for Forest Ecosystems (Eberswalde), in Figure 3 we present histogram of ammonia concentrations that have been measured at ICP Forests level II plots in 12 Federal States between 2001 and 2018. The numbers above the bars indicate the percent share of data in a concentration class. The grand mean across years and stations is 1.7 µg m^{-3}. While in the year 2009 ammonia has been determined at 38 stations, in 2001 and 2020 the gas had been measured at four respectively fifteen forest stations only. Over the years, no trend towards lower concentrations could be observed, which is in line with other studies from Europe and N-America.

Figure 3: Data evaluation (histograms) from long-term monitoring of atmospheric ammonia in German forests.

Insert shows a map with bars representing the concentrations that were determined in the year 2009.



Source: Own illustration after data from Thünen Institute of Forest Ecosystems, Eberswalde (pers. comm.).

We would like to once more refer the reader to the Conference Proceedings of a workshop which was held in the end of March 2022 in Dessau. Much of the information contained in this chapter and in Table 3 on the ammonia monitoring programs was shared from participants of this workshop. The proceedings (Franzaring & Kösler 2023) contain 19 short communications of oral presentations, which were given during the workshop. Many of them deal with the technologies involved in the monitoring and the scope of monitoring programs in various European countries and Northern America.

Table 3: Overview of ammonia monitoring programs using passive samplers

Country	Sampler type	Network name	N of sites[1]	Period	Network Details / Results	Sources
BE	Radiello®, blue diffusion barriers, miniDOAS		100 Natura2000 sites, miniDOAS at two sites	2015-2016	Country mean: 3.3 µg m^{-3} NH_3, in Western Flanders > 5 µg m^{-3} NH_3, no trends over time.	VMM (2017)
CAN-ONT	Ogawa	NAPS	74	2006-2007	Even in remote sites in CAN, 50% of the sites would exceed the critical levels.	Yao & Zhang (2013; 2019)
CH	Radiello®		13	since 2000	No reductions over time, different levels in different regions. All values exceed the 1 µg level, most the 3 µg critical level.	Seitler et al. (2018); Seitler & Meier (2022)
DE -Depo	FERM (IVL)		15 forest sites (in 2020)	since 2001	ICP-Forests Level II locations, bi-weekly. No decline in concentrations in German Forests. Grand mean: 1.9 µg m^{-3}.	Thünen Institute of Forest Ecosystems (pers. comm.)
DE-BW	FERM (IVL)		16[2]	since 2007	Selected urban and rural locations. Moderately high NH_3 at traffic sites.	LUBW (https://pd.lubw.de/10334)
DE-BY	Ferm (IVL)	LÜB, DBS	15	since 2011	Selected locations, four-weekly. Downward trend in cities, in rural areas increasing trend since 2017. Yearly mean levels up to 3 µg m^{-3} at rural sites, 7 µg m^{-3} at intensive agricultural sites.	LfU (2019) and LfU (pers. comm.)
DE-MV			10	2010	No trend study possible.	https://www.lung.mv-regierung.de/umwelt/luft/archiv/jb_2019_small.pdf
DE-NRW	Ferm (IVL)		5	since 2001	Selected urban and rural locations. No trend.	Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (pers. comm.)
DE-NI	Ferm (IVL)		19	since 2010	Selected rural locations. 0.7 to 13 µg m^{-3} ammonia. Two sites in industrial settings.	https://www.umwelt.niedersachsen.de/download/183444/Jahresbericht_2021.pdf
DE-SH			10	2008	No trend study possible.	https://www.schleswig-holstein.de/DE/fachinhalte/L/luftqualitaet/Downloads/Berichte/bericht_orient_messungen_NH3_SH_2011.pdf?__blob=publicationFile&v=1

[1] If measurements are still ongoing, number represents the current number of sites with operating ammonia measurements

[2] BW established 13 new sampling sites in 2021. They are not officially documented, therefore not reported here

Country	Sampler type	Network name	N of sites	Period	Results	Sources
DE-ST			7	since 2021/22	No trend study possible.	LUA (https://lau.sachsen-anhalt.de/luft-klima-laerm/lufthygienisches-ueberwachungssystem-luesa/)
DE-UBA	Denuder		7	2004	Network background sites of the German Environmental Agency	UBA (https://www.umweltbundesamt.de/das-uba/standorte-gebaeude#dessau)
F	Alpha, Delta		11	2009-2019	Selected agricultural and forest locations. High ammonia concentrations in Brittony.	Fauvel et al. (2019)
IRL	Alpha		12 Natura2000 sites	2017-2018	0.47–4.59 µg NH_3 m^{-3}. Agriculture is the main source and ammonia is related to ambient temperatures.	Kelleghan et al. (2021)
LUX	Gradko		4	since 2019	Mean concentrations vary from 5.5 to 19.2 µg m^{-3} at rural and agricultural sites.	Pers. comm. Trebs & Junk, Luxembourg Institute of Science and Technology (LIST 2022)
NL	Gradko, miniDOAS	MAN	Currently 310 measuring points at 105 sites, out of which 77 are Natura2000 sites, mini DOAS operated at 6 sites	since 2005	NH_3 concentrations significantly increased in easterly regions and went down in the west and in the south.	Lolkema et al. (2015); Noordijk et al. (2020); pers. comm. Jonkers and Bleeker
UK	Alpha, Delta	UK NAMN	74	since 1998	Reduction of 3% on national level, but ammonia increases in some regions.	Tang et al. (2018); United Kingdom Eutrophying & Acidifying Network (UKEAP), https://uk-air.defra.gov.uk/networks/
US	Radiello®, blue diffusion barriers	AMoN	50	since 2007	NH_3 concentrations have increased by 7% between 2008 and 2015 at 18 long-term sites.	Puchalski et al. (2011) and Yao & Zhang (2016; 2019)

3 Fumigation and Field study (WP2)

3.1 Objectives of the activities

In WP2 of the project, a fumigation study should be performed to establish dose-response relationships for representative N-sensitive plant species protected by the European Habitats Directive (Natura2000). We hoped to be able to derive dose-response functions for NH_3 for a number of vascular species. Latter reference values have mainly considered lower plant species (see chapter 2.2.2), but we wanted to at least grow four vascular plant species over a period of two years at low NH_3 concentrations. Nevertheless, we included a lichen species in the beginning of our trials, but the specimens soon died in the unnatural greenhouse environment.

It seemed technically challenging to investigate the concentration range from 0 (control) to 10 µg m^{-3}, since the ambient air in Hohenheim has an average concentration of about 3-5 µg m^{-3} according to own ammonia measurements (for measurements in Germany see Table 3). We therefore did not strive for reaching a concentration of zero, which would mean to have air filters in the fumigation unit. The only feasible way to generate ammonia depleted air relies on H_2SO_4 scrubbers that had been developed for animal husbandry. The approach would nevertheless not scavenge all ammonia and enrich the air with H_2S and SO_2, so that the plants would have to deal with another stressors, i.e. highly phytotoxic gases.

We therefore decided to "drive" the control treatment with ambient air and refrained from using activated carbon filtered or cleaned air with an acidic scrubber. It must also be noted that even when using ammonia-free air, small amounts of NH_3 will also be released from the soil and the plants themselves. The ammonia flow and the direction in or out of the plant depends mainly on the NH_4^+ concentration in the apoplast and the temperature. The so-called "compensation point" is being reached when the NH_3 concentrations in the outside air and the substomatal cavities are the same (see chapter 4.1.1).

In order to maintain a constant flow of NH_3 from the air into the plant, only substrates with low nitrogen contents, preferably non-fertilized, natural substrates had to be used. We therefore worked with potted plants and only used one plant per pot since we were not addressing the competitive interactions, which drive the responses in the natural vegetation. In the natural plant community, sensitive species will sooner or later be out-competed by the faster growing species that can assimilate more nutrients than the slow growing species. At deposition velocities of 1 cm s^{-1} typical for low growing vegetation and independently from the nutritional status (i.e. compensation points) of the plants on site, ammonia concentrations of 10 µg m^{-3} will result in a nitrogen deposition of almost 25 kg NH_4-N ha^{-1} a^{-1}. Loads of such a magnitude will already be relevant to non-agricultural ecosystems and indeed, in ecosystems prone to high nitrogen deposition, e.g. acidic grasslands and heaths, N sensitive species have already become rare. Vegetation changes and biodiversity losses due to N deposition were the reason, why the contracting authority wanted us to address N sensitive species in pot experiments.

We hypothesized that at low concentrations of ammonia fumigation most slow growing nitrogen sensitive species grown as single plants would not be able to much increase their biomass and that adverse phytotoxic responses would be absent at ammonia levels below 10 µg m^{-3}. As stated before, we focused on single plants in pot experiments and strived for the derivation of dose-response values for individuals.

Besides a fumigation study in the first year in a closed environment, we also worked outdoors and established a field gradient study. In the lab, the harvested biomass of two of the selected perennial species were subjected to nitrogen and stables isotope analyses. All the activities are described in greater detail in the following sub-chapters.

3.1.1 Selection of plant material

3.1.1.1 Perennials used for the fumigation and the field study (first and partly second year)

Realizing the pivotal importance of the varying nutrient ecology of Central European plant species, we came up with an initial list of around 25 perennial taxa from nitrogen poor habitats, which under competition and in their realized niche with other plants should show negative responses to prolonged nitrogen inputs.

Based on the selection of potential candidate species, in the beginning of the project, contacts were made to various universities, botanical gardens and scientific networks to get hold of seed material from plant species characteristic for N-sensitive habitats, which are listed in the Natura2000 annexes or in the red data book as highly endangered or for which Germany has a special responsibility. Several lists[3] were screened to identify interesting species and to get preliminary information on their availability, germination and growth conditions.

Table 4: List of perennial plant species finally selected for the fumigation study

The 12 species were sown on the 3rd of June 2019. Reasons for the selection, the protection status and the ecology of the species are given in the text. “L”, “T”, “K”, “F”, “R”, “N” refer to the ecological indicator values by Ellenberg et al. (2001). Life forms: “H”-hemicryptophyte, “C”-chamaephyte and “G”-geophyte. Leaf types: “I”-evergreen, “W -green during the winter, “S”-green during the summer, “V”-green before the summer. Note: species in black were finally used in the two-year fumigation study. However, we used *P. vulgaris* instead of *P. grandis*, since latter species did not germinate in a high enough quantity.

Scientific Name Author	English Name	Priority program Bot. Gard.	Natura 2000 Ann. V	WIPs-DE II List	Red Data Book	L	T	K	F	R	N	Life form	Leaf type
Plant species 2020													
Antennaria dioica (L.) Gaertn.	Pussytoes	•			•	8			4	3	2	C	I
Arnica montana L.	Wolf's Bane	•	•	•	•	9	4	4	5	3	2	H	S
Carex arenaria L.	Sand Sedge			•		7	6	2	3	2	2	G, H	I
Dianthus deltoides L.	Maiden Pink	•				8	5	4	3	3	2	C, H	W
Koeleria glauca (Spreng.) DC.	Blue Hair Grass	•			•	7	7	7	3	8	1	H	S
Molinia caerulea (L.) Moench	Purple Moor Grass					7	x	3	7	x	1	H	S
Pulsatilla vulgaris Mill	Pasque Flower	•				7	6	5	2	7	2	H	S
Plant species 2021 (additional)													
Dianthus gratianopolitanus Vill.	Cheddar Pink	•		•	•	9	7	4	2	7	1	C	I
Bromus hordeaceus L.	Soft Brome					7	6	3	x	x	3	H, T, A	W
Erodium cicutarium (L.) L'Hér.	Restem Stork's Bill					8	6	5	4	x	x	H, T, A	W
Iberis amara L.	Bitter Candytuft				•	7	7	2	4	8	3	H, T, A	W
Legousia specul.-veneris (L.) Chaix.	Venus's Looking Glass	•			•	7	7	4	4	8	3	T, A	S
Trifolium arvense L.	Rabbitfoot Clover					8	6	3	3	2	1	H, T, A	W

When talking about the scope of the present project to potential seed suppliers it became clear, however, that it would be difficult to obtain seeds from a special geographical origin in a high

[3] Lists of highly endangered species for which Germany has a high responsibility can be found under https://www.wildpflanzenschutz.uni-osnabrueck.de/wp-content/uploads/2019/07/92_Pflanzenverantwortungsarten.pdf and http://www.ex-situ-erhaltung.de/pflanzenarten/

enough quantity. Most of the approached scientists were not even willing to supply seeds from their *ex-situ* conservation programmes since for them it would be more important to use them in practical vegetation regeneration than in experiments like ours. We thus decided to purchase seed material from the commercial seed supplier Jelitto® Perennial Seeds although the company is unable to provide information where the seed material originally stemmed from. The company propagates the seeds itself and operates after high standards for avoiding outbreeding, seed cleaning and storing the germplasm. Only for *Arnica montana* L., we were able to get hold of germplasm from two natural populations from the Rhön mountains.

After merging the lists of Natura2000 annex species, red data books and the lists of the above-mentioned German priority programmes we collated information on the ecology and the life forms of the plant species. We decided to exclude woody species and focussed on perennial grasses and herbs only, since the experiments should be performed over two vegetation periods. An important criterion was that all species should be from nutrient poor habitats. Species should thus have Ellenberg N-indicator values of <= 2. On the other hand, we wanted to ensure that species stem from both, acidic and basic habitats and are from diverse taxa. Table 4 shows the final list of 12 species we ended up with and from which seed material was purchased and sown.

In the following paragraphs, short species accounts are given for those taxa that were ultimately used in the two-year experiments in order to describe their biology, ecology and representativeness for certain Natura2000 habitat types. Information on the attributed Critical Level for several habitat types (currently LRT 1110 bis 5130) is available in Ssymank et al. (2021, 2022).

***Antennaria dioica* (L.) Gaertn.**

Pussytoes (Asteraceae) is an endangered herbaceous plant species in Germany (red list 1996, level 3+) and whole central Europe. It grows in lowlands as well as in alpine regions, reaching altitudes over 2400 m a.s.l.. However, (sub) alpine sites are most commonly populated. *Antennaria dioica* preferably grows on lime-deficient, sandy-loamy, nitrogen-poor sites with lower pH and is a character species of different *Nardus* dominated associations. The main Natura2000 habitat type is “species-rich Nardus grasslands” (6230*) with an estimated critical level of 2-3 µg m^{-3} according to StickstoffBW (2019) and Ssymank et al. (2022). The plant species is particularly protected in the Federal Nature Conservation Act. Reasons for the species’ endangerment are mainly soil eutrophication due to fertilization and nitrogen deposition.

***Arnica montana* L.**

Wolf’s Bane (Asteraceae) is endangered in Germany (red list 1996, level 3), but not in whole central Europe. It thrives on acidic, lime-deficient and nutrient-poor sites such as dwarf-shrub heaths, *Nardus* grass grasslands and light forests. It is mainly found in alpine mountain regions, but also in the lowlands. Natura2000 habitats with *Arnica montana* are amongst others “European dry heaths” (4030, with an attributed critical level of 2-3 µg m^{-3}), “species-rich *Nardus* grasslands” (6230*, 2-3 µg m^{-3}) as well as “Alpine and Boreal heaths” (4060, no estimated critical level available). Besides the adverse effects of eutrophication and acidification (Dueck and Elderson 1992) and its excessive use as a medicinal plant, the species strongly responds to summer drought (Stanik et al. 2020).

The species is particularly protected in the Federal Nature Conservation Act. Main reasons for this species’ endangerment are habitat eutrophication due to fertilization and widespread N-deposition. We used two varieties, one from the Rhön region (Weiherberg, Plus Code GW96+MQ Hilders) and a commercial origin from the company Jelitto®.

***Carex arenaria* L.**

Sand Sedge (Cyperaceae), is neither endangered in Germany, nor particularly protected by law. However, it is a character species for special nitrogen-poor sites. This sedge is an evergreen pioneer plant when it comes to colonizing sandy sites such as dunes. It produces long stolons (10 meters) beneath the surface and forms regular aboveground sprouts. This is how it contributes to stabilizing the sandy soil. It occurs for example in the Natura2000 habitats 2330 ("Inland dunes with open *Corynephorus* and *Agrostis* grasslands" with an attributed critical ammonia level of 1-2 µg m^{-3}) and 2130 ("Fixed coastal dunes with herbaceous vegetation (grey dunes)", no estimated critical level available). *Carex arenaria* is a typical species found in the *Corynephorion canescens* associations.

***Dianthus deltoides* L.**

Maiden Pink (Caryophyllaceae) is not endangered in the whole of Germany, but is mentioned on the red list of some federal states and is therefore particularly protected by the Federal Nature Conservation Act. The herbaceous species grows on lime-deficient, slightly acidic sites such as nitrogen-poor silicate grasslands, dry grasslands and dwarf-shrub heathlands.

It is a typical species of the Natura2000 habitat 2330 (Inland dunes with open *Corynephorus* and *Agrostis* grasslands) with both, a critical ammonia level of 1-2 µg m^{-3}.

***Koeleria glauca* (Spreng.) DC**

Blue Hair Grass (Poaceae) is strongly endangered in Germany (red list 1996, level 2), but not in the rest of Europe. It mainly occurs in (semi) dry grasslands, on sandy, extreme nutrient-poor and base-rich sites and is a character species of the FFH habitat 6120*, "Xeric sand calcareous grasslands (*Koelerion glaucae*)". The preliminary empirical critical ammonia level for these habitats is 1-2 µg m^{-3}. The species is mainly endangered due to urban development, habitat destruction and replacement. However, it is not particularly protected by the Federal Nature Conservation Act.

***Pulsatilla grandis* Wender**

Pasque Flower (Ranunculaceae) is an herbaceous species strongly endangered in Germany (red list 1996, level 2) that is strictly protected by the Federal Nature Conservation Act. In Germany, it only occurs in Bavaria and Thuringia, mainly on extreme nitrogen-poor, calcareous (semi) dry grasslands, reaching sub montane altitudes. It is often addressed as being the same species like *Pulsatilla vulgaris* (Kaligaric et al. 2006) which typically grows in the Natura2000 habitat 6210* ("Semi-natural dry grasslands and scrublands on calcareous substrates in the order of *Festuco-Brometalia*, important orchid sites") with a preliminary critical ammonia level of 2-3 µg m^{-3}.

After keeping the seeds at 4°C in a refrigerator for a month to break seed dormancy, sowing of the 12 species took place on 3rd of June 2019 on three types of substrates to meet the ecological requirements of the different species. An acidic peat based (pH 4.95) substrate was used for *A. montana*, *A. dioica* and *G. pneumomanthe* (later excluded because the quanity of germinated seeds was too small). An acidic sandy substrate (pH 3.83) was used for the heath species *C. arenaria, D. deltoides* and *J. montana* (also not sufficient seedlings) A mixture of peat-based flower earth: river sand: cleared lime (1:1:0.3, pH 7.27) was used for the rest of the species, which are typical for basic soils.

Seeds were sown in plastic trays and were transferred to propagation trays (77 cups per tray) two weeks after emergence. Watering was done on demand using rain water. Once a dense root system had been formed, the plantlets were transferred into individual pots. These pots were filled with substrates that were blended with natural soils in order to simulate quasi-realistic edaphic conditions. For Arnica we used a soil from a meadow in the Hunsrück region (Züsch, Rhenanian Slate Mountains), where the plant grows. For the heath species, we gathered top soil from the Natura2000 Senne (Hövelhof) inland sand dunes. We had to get official allowances from the Hunsrück-Hochwald National Park Administration and the Senne regional bodies to extract soil. For the other species, we used a mixture based on calcareous Rhine sand.

Finally, we had five plant species available in a high enough quantity, namely *A. montana* (two origins), *A. dioica*, *C. arenaria*, *K. glauca* and *D. deltoides*. While the first five species were grown from seeds, the pink plants were propagated vegetatively from mother plants from a private garden. Because we wanted to have a limestone preferring species in our experiments, but *Pulsatilla grandis* did not germinate in a high enough quantity, we decided to buy 100 one-year old plants of *P. vulgaris* from a plant supplier.

Furthermore, we propagated clones of the ***Molinia caerulea*** **(L.) Moench** (Purple Moor Grass) in our institute garden, which were also used in the fumigation experiment. The ramets stem from a study on CO_2 responses of the species as modified by nitrogen supply (Franzaring et al. 2008). The original clone came from a dry heath Eastern Westfalia. The grass is a good indicator of eutrophication and we wanted to test whether it reacts on ammonia and whether it gets a higher competitive ability when more nitrogen is available. Various studies were previously able to show that N-deposition was the main driver for its increased frequency (Aerts and Caluwe 1989; Berendse et al. 1994; Hogg et al. 1995; Damgaard et al. 2017). The grass species could thus be a suited active bioindicator to determine the deposition of N containing pollutants. Indeed, Laffray et al. (2010) were able to show that the grass showed increased growth from the input of NO_x and NH_3 along French roads. The method they implied was originally developed at the Institute of Landscape and Plant Ecology of the University of Hohenheim (the contractor), but in none of our previous studies with *M. caerulea* (Kopsch 2011) we could observe a stronger growth or higher N contents in pre-cultured and weighted plants at elevated concentrations of ammonia. In order to clarify whether the grass is stimulated by ammonia, we wanted to include pre-cultivated specimens of the species in our fumigation experiments.

3.1.1.2 Plants used for the field exposure (only second year)

Since in the fumigation experiment performed in greenhouse chambers in the first year (2020), plants were exposed to very high temperatures and did not show the same phenology like the plants in the field, we agreed with the contractor to continue our experiment in the field in 2021. The experiment was performed at the Hohenheim university farm Unterer Lindenhof (ULI), which is described in detail in Chapter 3.3.

Because we were not sure, whether the perennial plants we had cultivated in the greenhouse under extreme temperatures would show a normal development in the field, we stipulated to include additional species in the experiments. These had to be pre-grown in the early spring 2021 before they were transferred to the field in the beginning of May 2021. Without knowing how the vegetation period respectively the climate would develop, we started with the re-location of the experiment to the field. For the additional plant species chosen, we decided to not test the drought reaction, but to focus solely on the ammonia treatments. Therefore, we only needed 50 plants per species (ten replicates per treatment). The perennial species *Dianthus gratianopolitanus* which was also selected for the field study had also not been subjected to drought stress in the previous season. In the following, information on each additional species is given.

Erodium cicutarium

We originally intended to use *Erodium lebelii*, but could not get hold of seeds. We therefore decided to purchase the closely related, but very common *Erodium cicutarium* (Geraniaceae). The species is neither endangered, nor protected by law in Germany. It is widespread throughout whole Europe and behaves indifferently with regard to nitrogen input (N=X). We thought it still interesting to observe the species' reaction to the different ammonia concentrations and to compare them to the reactions of the other, potentially more sensitive, species.

E. cicutarium was the first annual species to be transferred to the ULI farm and to be exposed to the different NH_3 concentrations. The annual species needed some time to adapt to the new substrate and pots before starting to grow.

Bromus hordeaceus

Bromus hordeaceus is a grass species that is not endangered in Germany. We chose the species because of its rather low Ellenberg N value of 3 and because we wanted to include another grass in the experiment. *B. hordeaceus* plants were transferred to ULI on 6 May 2021 and it was the only species with three plants per pot due to its small size.

Iberis amara

Iberis amara is a species deemed to be extinct in Germany. It has an Ellenberg N value of 3 and is used as a medicinal plant due to high levels of the bitter cucurbitacin. It used to grow on nitrogen-poor fields. *I. amara* plants were re-potted and then transferred one week later, on 12 May 2021.

Trifolium arvense

Trifolium arvense is neither endangered nor protected in Germany, but is an indicator plant for extreme nitrogen-poor sites (Ellenberg N=1). The legume mainly grows on dry and semi-dry grasslands.

Legousia speculum-veneris

Legousia speculum-veneris is strongly endangered in Germany, but not protected by national or international law. It naturally grows as a weed on extensive calcareous fields (N=3).

Dianthus gratianopolitanus

Plants were brought to the ULI farm on 12 May 2021. *D. gratianopolitanus* is endangered and protected in Germany and is an indicator plant for extreme nitrogen-poor soils (N=1). It mainly occurs in dry/semi-dry grasslands.

3.1.1.3 Lichens

We originally also intended to use lichens in our fumigation experiments since some organisms of the group of lower plant species are recognised to be responding strongly to the input of nitrogen by N-containing gases. Lichen monitoring is thus very often used in studies seeking to determine the effect of eutrophication (e.g. the N-lichen app developed by CEH). However, we must point to the fact that there is a large number of species of lichens and many of them are nitrophilous but rare.

Lichens are composite organisms consisting of at least one photobiont (an alga or a cyanobacterium), which generates energy and providea assimilates to a partner, namely a fungus, which in return provides a special microclimate for the algae or cyanobacteria to grow. The special symbiosis is very sensitive to air contaminants (the extent differing from species to species) and is therefore often used in air monitoring projects (chapter 1).

For our work we wanted to expose a presumably nitrogen sensitive lichen species (*Evernia prunastri* (L.) Ach.) to the different ammonia concentrations in the same compartments where the above mentioned perennial higher species were kept. The lichen species has been used in similar ammonia experiments before and is described as a suitable bioindicator (Munzi et al. 2014; Munzi et al. 2019). Therefore, branches with the fruticose lichens had been collected in early 2020 in the western Saar-Hunsrück Nature Park region in the communities of Greimerath and Britten (Rhineland-Palatinate and Saarland).

A construction for the exposure of the branches had been designed and built to fix small branches (Figure 4). Due to the expertise required for the identification of the complex organisms, an external lichen specialist from the Museum of Natural History Stuttgart, was asked to help distinguish various species we had in mind before the final selection was made. During the two years of the experiment, we wanted to measure the growth/expansion of the lichens and determine the chlorophyll fluorescence, latter of which is an indicator for the photosynthetic activity and therefore the fitness of the photobiont.

Figure 4: Lichen-hosting branches with the fruticose lichen species *Evernia prunastri*

Lichen material was collected in the western Saar-Hunsrück Nature Park. The fruticose lichen visible in the picture is the N-sensitive species *Evernia prunastri*. Sixteen branches, hosting one to several individuals, were chosen per ammonia treatment and fixed to a wooden construction (right). Photos stem from March 2020 when lichens still looked healthy.

Source: Own photographs, the author.

Sixteen lichen-hosting branches were chosen per greenhouse compartment and representatives from thinner to thicker branches and smaller to larger lichens were selected to account for differences in initial size. The five constructions with the lichens were exposed to the chambers in May 2020 and were watered every three days with rain water using five lifts per rack from a spray flask.

Only six months after the collection of the lichens from the wild and after three weeks in the hot greenhouse environment, most of the specimens appeared to be inactive and in July 2020 all of the lichens had died. Disappointingly, we could not continue with the fumigation of lichens.

3.1.2 Cultivation of the plants

Following tables (Table 5, Table 6) show how the two sets of plants were cultivated and when they were exposed in the fumigation (2020) and field experiments (2021).

Before the start of the fumigation experiment, the pre-cultivated perennial plants were all kept in an unheated greenhouse during the winter 2019/2020 and were watered on demand with rain water. With the beginning of plant development in spring 2020, we selected 100 of the fittest plants of each species and labelled them. We took first measurements such as height or leaf number and fertilized first species with a low amount of ^{15}N marked fertilizer solution (corresponding to 5 kg N ha^{-1}). Before transferring the plants to the greenhouse facilities of the University Hohenheim, called Phytotechnikum (PHT), soil samples were taken to determine the original ^{15}N signature. With the change of ^{15}N signatures over time, we hoped to trace the NH_3 plant uptake via the atmosphere and performed these isotopic analyses on only two species, namely *A. montana* and *M. caerulea*.

Table 5: Information on the substrates and procedure for the cultivation of perennial plants that were used in the fumigation and field experiments (2020 and 2021)

Plant species (2020)	*Antennaria*	*Arnica (both)*	*Carex*	*Dianthus d.*	*Koeleria*	*Molinia*	*Pulsatilla*
Substrate mixture (Volume parts)							
Rheinsand (contains a bit chalk)	2	2	1		1	1	1
Seedling Substrate (Klasmann)	1	1	5,25	3	1	1	1
Sennesand (acidic)			0,75	1			
Hunsrück soil	1	1					
Sowing	03.06.2019	03.06.2019	03.06.2019	03.06.2019	03.06.2019		
Re-potted in 1l Göttinger pots	09.04.2020	02.04.2020	29.04.2020	16.04.2020	19.01.2020	18.02.2020	20.01.2020
Fertilized and ^{15}N addition	23.04.2020	08.04.2020	07.05.2020	07.05.2020	03.03.2020	01.04.2020	03.03.2020
Plants per pot	1	1	1	1	1	1	1
Exposure date PHT 2020	30.04.2020	30.04.2020	30.04.2020	30.04.2020	30.04.2020	30.04.2020	30.04.2020
End of Exposure 2020	14.10.2020	14.10.2020	14.10.2020	14.10.2020	14.10.2020	14.10.2020	14.10.2020
Duration of Exposure (days)	167	167	167	167	167	167	167
Exposure date ULI 2021	25.03.2021	06.05.2021	24.06.2021	30.06.2021	25.03.2021	28.05.2021	18.03.2021
End of Exposure 2021	07.07.2021	13.10.2021	06.10.2021	25.08.2021	13.10.2021	29.09.2021	06.10.2021
Duration of Exposure (days)	104	160	104	56	202	124	202

Table 6: Information on the cultivation of annual plants and *D. gratianopolitanus* that were used only in the field experiments of the second season (2021)

Plant species (2021)	*Dianthus g.*	*Bromus*	*Erodium*	*Iberis*	*Legousia*	*Trifolium*
Substrate mixture (Volume parts)						
Rheinsand (contains a bit chalk)	1	1	1	1	1	1
Seedling Substrat (Klasmann)	1	1	1	1	1	1
Sowing	06.06.2020	09.02.2021	09.02.2021	09.02.2021	09.02.2021	09.02.2021
Re-potted in 1l Göttinger pots	26.08.2020	29.03.2021	15.03.2021	27.04.2021	11.05.2021	03.05.2021
Plants per pot	1	3	1	1	1	1
Exposure date ULI 2021	12.05.2021	06.05.2021	18.03.2021	12.05.2021	09.06.2021	24.06.2021
End of Exposure	18.08.2021	16.09.2021	30.06.2021	21.07.2021	15.07.2021	22.09.2021
Duration of Exposure (days)	98	133	104	70	36	90

Potting: In March and April 2020 all plants were potted into 1L round Göttinger plastic pots (truncated cones) that were filled with potting mixtures meeting the nutrient demand of different species. Before the onset of the fumigation, plants were fertilized with ^{15}N labelled

NH_4NO_3 nutrient solution corresponding to 5 kg N ha^{-1}. Plants were progressively moved into the greenhouse compartments in May 2020 according to their different phenology. Also, the annual plants that were only used in the field study in 2021 were potted in 1L Göttinger plastic pots to have a comparable soil volume.

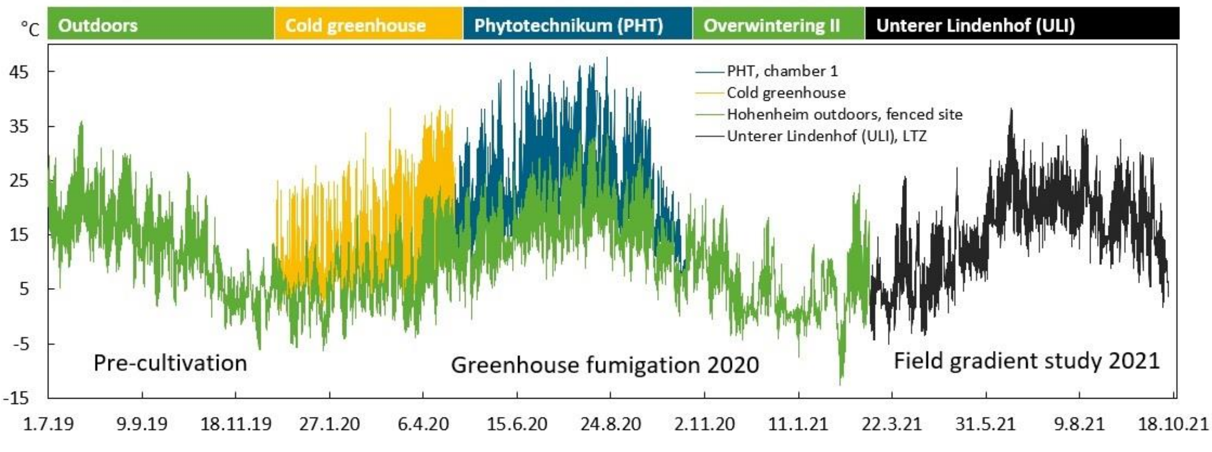
Plant protection measures: Before the perennial plants were transferred to the greenhouse, aphids were detected at most *Pulsatilla vulgaris* and *Arnica montana* Jelitto plants. In order not to carry pests into the new greenhouse complex and in order not to harm plants via pests right before the onset of the experiment, we decided to treat them with a low amount of the pesticide Biscaya® (active ingredient: thiacloprid) on the 24th of April 2020. All treated plants handled the treatment very well and were not visibly harmed by the product.

Then, plants were finally transferred to the PHT on the 30th April 2020 and allocated to the five chambers. Plants were separated in normal and dry treatments to facilitate watering and were randomized within these treatments. Unfortunately, another pest was detected on *Arnica montana* (both origins) plants shortly after. Thrips (*Thysanoperans*) infected most of the *Arnica* plants and therefore, predator mites (*Amblyseius cucumeris* and *Amblyseius barkeri*) were added to the plants in bran substrate. Furthermore, papers containing larvae of lacewings (*Chrysoperla carnea*) were brought out in every compartment to prevent further aphid and other pest infestation. We strived to prevent the use of chemical plant protection products and we wanted to draw on biological pest control as far as possible in the greenhouse facilities.

In the cold house as well as in the PHT and later in the field, weeding was conducted manually when plants were watered or measurements were taken.

Figure 5: Temperatures plants were exposed to during the whole experiment

Individuals were grown in a pre-culture outdoors (temperature green line) in Hohenheim during the summer 2019. They were then moved into a cold greenhouse for the first winter (orange line) and after re-potting they were fumigated with ammonia in the Phytotechnikum (light blue line) during the summer 2020. In fall 2020, plants were left overwintering outdoors for the second winter (green line) and after March 2021 they were transferred to Unterer Lindenhof (ULI, black line) for the field gradient study.



Source: Own illustration, University of Hohenheim.

3.1.3 Climate throughout the whole plant study

The experiments ran from 1 July 2019 to 13 October 2021 (28 months). After the selection and pre-cultivation of initially twelve perennial nitrogen sensitive plant species in the summer 2019, plant trays were moved into a cold greenhouse in the winter 2019/20 on the campus of the

University of Hohenheim to make sure that plants were not exposed to frost. As can be seen in Figure 5, temperatures in the cold greenhouse (orange lines) were about 6°C higher than outdoors (green line). It may thus well have been that vernalisation (flower induction in plants after a prolonged cold period) of some of the chosen plant species was not complete in the first experimental season. During the fumigation experiment, mean temperatures in the greenhouse (blue lines) were about 8°C higher than outdoors (green line). We assume that temperatures were much too high for some of the selected native central European species, since most of the species remained in the vegetative phase.

In order to assure the plants a more natural growth climate, we transferred all the plants to an outdoors field site after the greenhouse fumigation. The winter 2020/21 was slightly cooler (green line) than during the last decade, but most of the plants withstood the frost spell in February 2021. In February and March 2021 five annual species were grown, which together with the perennial species should be moved to the ammonia gradient study at the Unterer Lindenhof (ULI). As can be seen in the figure, temperatures in the field (2021, black line) were around 8°C lower than those recorded in 2020 in the greenhouse (blue line). Further comments on the climate during the greenhouse and the field study will be made in the following chapters.

3.1.4 ^{15}N labelling and analyses

In nature, nitrogen exist in form of two stable isotopes, ^{14}N and ^{15}N. The isotope with the larger proportion of the natural isotopic composition is ^{14}N with 99.636 %. In contrast ^{15}N has a proportion of 0.364 %. Therefore, assessing natural or artificially changed proportion of the two N-isotopes in substrate and plant tissues allows to assess preferred uptake pathways of plants. Positive or negative deviations (fractionizations) from these defined isotopic ratios (i.e. the natural abundance) in environmental or biological samples are expressed as delta (δ) values and are given in per mille (‰).

There have only been a limited number of studies to date, in which ^{15}N labelled ammonia has been used to investigate the uptake, metabolic fate and nutritive action of the gas in plants. Since only a few labs in the world can generate, control and monitor the isotopic signatures of technical gases, the use of such ammonia will have to be restricted to short periods and small chambers only. These experiments can thus not be representative for complete life cycles of plants and complex interactions in ecosystems with other nitrogen sources and are restricted to a short duration and plant parts, which are exposed to ammonia.

An example is Adriaenssens et al. (2012), who at three times in the season (June, August and September) for 2 h exposed branches of four tree species to ^{15}N labelled ammonia representing concentrations between 5 and 100 ppb (3.4 to 69.5 µg m^{-3}, conversion for 25°C after http://www.apis.ac.uk/unit-conversion). In the study there was no documentation on where the labelled ammonia stemmed from and how it was generated and mixed. Due to the short exposure, there were only very weak relationships between ammonia and plant nitrogen contents, but deciduous species took up more ammonia than pine.

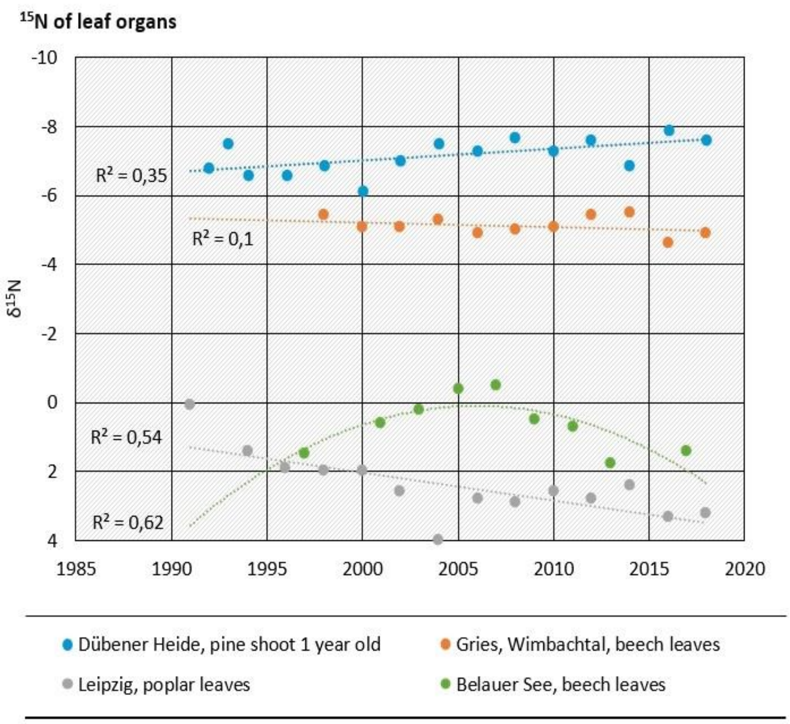
Also, Huang et al. (2020) used ^{15}N labelled ammonia that was not supplied as a gas, but which was generated, i.e. stripped with NaOH from a labelled ammonium sulphate solution supplied in beakers in closed chambers. The focus of that study was to test how much of (soil) released ammonia could be taken up during 24 hours by tomato plants. However, ammonia concentrations and ^{15}N signatures of the generated ammonia were not measured. Nevertheless, the authors suggested from ^{15}N analyses of the plant material that 80% of the volatilized ammonia was fixed by the leaves, whereas 20% were remobilized to other plant parts. It must be noted that these values will be much lower in the field than in closed growth chambers because of the diffusion of ammonia into the open air.

In order to investigate and at best, trace how much of the nitrogen incorporated in the plant stems from the airborne supply of ammonia, we intended to use changes in the isotopic signatures over time, i.e. to perform a ^{15}N dilution experiment.

It is well known that epiphytic lichens in livestock dominated areas of Northern Germany have a much lower and more negative $\delta^{15}N$ than those in the Ruhr-Rhine area, where oxidized forms of nitrogen prevail (Boltersdorf and Werner 2013). The reason is that volatilization of NH_3 from manure strongly discriminates against the heavier ^{15}N, i.e. more ^{14}N is present in the air. The process depletes the $^{15}NH_3$-N by 24.5 ‰ in the air and enriches the unreacted NH_4^+-N by ^{15}N in the liquid manure (Bedard-Haughn et al. 2003). It may be suggested that ^{15}N depleted ammonia will then lead to lower $\delta^{15}N$ signatures in plant matter that during its life has absorbed ammonia. This should be more pronounced in N demanding plants (low compensation point) than in well fertilized plants that will be a source for ammonia (bi-directional flux, see Massad et al. 2010).

Figure 6: Changes in $\delta^{15}N$ signatures (in ‰) of foliage from different tree species collected at the permanent monitoring plots of the German Environmental Specimen Bank (Umweltprobenbank des Bundes, data retrieved from https://www.umweltprobenbank.de/de)

Negative values may be related to the influence of regional ammonia (where less of the heavier isotope is present), while positive values suggest the deposition of oxidized nitrogen species (with relatively more of the heavier isotope).



Source: Own illustration after data from the UBA Environmental Specimen Bank, University of Hohenheim.

^{15}N analyses have also been made, using historical samples from the German environmental specimen bank (Umweltprobenbank des Bundes, UPB). These data show that different tree species lead to different fractionation (Figure 6). Conifer needles show a stronger discrimination of ^{15}N than deciduous species, but the emission sources and their composition in the different regions seem to play a bigger role than the identity of species. However, evaluations of the data and temporal trends have not been made to date, but data suggest a stronger importance of reduced nitrogen at Dübener Heide and more oxidised N in Leipzig. The use of ^{15}N in field approaches to separate between oxidised and reduced forms of nitrogen has also been proven useful by Xu et al. (2015).

Before the onset of the fumigation, we fertilized the soil of the perennial plants with a double labelled $^{15}NH_4{}^{15}NO_3$ salt (10.28 atom% ^{15}N). Mixing the ^{15}N labelled fertilizer with unlabelled ammonium nitrate yielded an excess of $\delta^{15}N$ of +200‰ compared to the natural abundance that should be clearly different from the discrimination of the heavier isotope due to ammonia volatilization. Mixing of the sources of airborne nitrogen (presumably more negative isotopic signature, more ^{14}N) with soil borne (more $\delta^{15}N$, more positive δ) should over time lead to a ^{15}N dilution. This should enable us to differentiate the N uptake between the different ammonia treatments and possibly different plant species. The quantity of labelled nitrogen supplied to each pot initially should amount to a low N availability of 5 kg ha^{-1} in order to keep the system at the N-limited side. At a higher fertilization level, plants would probably show no need to acquire extra nitrogen from the ammonia available via the air.

In order to get information on the initial ^{15}N soil signatures that resulted from adding the labelled fertilizer, we took soil samples from each of the plant species before the onset of the fumigation. ^{15}N analyses and the determination of N concentrations and contents of plant and initial soil material and the determination of dry biomass in the end of each of the seasons should then unravel how much N came into the system via the ammonia exposure.

^{15}N signatures of the ammonia used in the experiment

Ideally, information on the original ^{15}N signatures of the liquid ammonia should be available that would be used in the fumigation experiment. However, the manufacturers do not need to provide this information to users. At the same time, no guarantee can be given by the producers that over time the isotopic signatures of products will stay the same since the synthesis of chemicals and the supply chains will vary with prices and availability on international markets.

We discussed the topic with Dr. Wolfgang Armbruster from the Institute of Food Chemistry of the University of Hohenheim and were grateful that he performed the isotopic analyses involved before we started with the fumigation experiments. Isotope-ratio mass spectrometry (IRMS) and various standards served to determine the ^{15}N signatures of NH_4^+ that was precipitated from ammonia evaporating from 10% NH_4OH solutions. The ammonia evaporating from the liquid ammonia was also collected on Radiello® passive samplers to get information on the ^{15}N signatures. However, we could not study ^{15}N-signatures of the tested liquids since the Hohenheim IRMS system can only handle solids. After collection of ammonia, the passive samplers, i.e. microporous PE rods, were ground to a fine powder and subjected to EA-IRMS (element analyser IRMS).

In a pre-test for the selection of a liquid ammonia for our fumigation experiments, we checked different products (Meffert, VWR, Alfa Aesar and Hach) of 10% liquid ammonia and investigated the isotopic signatures of the ammonium that was captured on passive samples upon the release of ammonia using IRMS. 5 mL of liquid ammonia were put into labelled Erlenmeyer flasks and a Radiello® cartridge was put into a 2 mL glass vial into the flask so that the cartridge remained dry. A watch glass was put on the top of the Erlenmeyer flask to reduce the outgassing of ammonia.

After seven days, the Radiello® cartridges were taken out of the Erlenmeyer flasks and their ^{15}N signatures were determined using IRMS again. On average, $\delta^{15}N$ signatures increased to over 90 ‰. Obviously, lighter NH_3 evaporated from the samplers and left back strong positive signatures created by the higher abundance of ^{15}N. The samplers had accumulated similar amounts of nitrogen at their matrices and there were only slight differences in the ^{15}N signatures that resulted from the evaporation of liquid ammonia from different suppliers. NH_4OH solution from Hach contained more ^{15}N than that of Alpha Aesar. Obviously, the isotopic discrimination due to adsorption, absorption and re-volatilisation at/from matrices like passive samplers and plant leaves (bi-directional fluxes) is more important than the initial ^{15}N signature of liquid ammonia. In order to nevertheless guarantee consistency in ^{15}N signatures over time, we used the same chemical product (9.5% liquid ammonia, Meffert Bad Kreuznach) during the experiment and purchased a larger quantity in the beginning of the experiment.

For the generation of different ammonia concentrations in the air of the four greenhouse compartments with addition of NH_3, we used diluted liquid ammonia that was led at a constant flow rate into the chambers via a peristaltic pump. The concentration and volume of realized/vaporized ammonia had to be adapted to the air exchange rates of the compartments (see chapter 3.2.1 for fumigation approach).

3.2 The chamber study (2020)

According to the original tender and our proposal, the fumigation should be based on low concentrations (ideally five levels from 0 to 10 µg m^{-3}), long exposure (two seasons) and pot grown plants of different taxa relevant to nature protection. Furthermore, a second environmental factor should be addressed in the project to study the interactions between eutrophication and climatic change.

Experiments were performed in the new Phytotechnikum Hohenheim (PHT) greenhouse. Five compartments of 14 m^2 resp. 70 m^3 each had been booked via the Service Unit Hohenheim Greenhouses (SHG). Space had been granted for our trials from July 2019 to October 2022. Staff from the Service Unit had to be involved for technical support and assistance with the cultivation and plant protection of the plants during the weekends. The location is indicated in Figure 7.

3.2.1 Experimental setup, methodology of ammonia fumigation

In order to avoid ammonia peaks and to protect people and material from accidental releases, we refrained from using pure technical ammonia. Instead, a time-controlled evaporation of diluted solutions was used to tune in low concentrations of ammonia in four greenhouse compartments. The fifth greenhouse unit was used as a control, where no ammonia was applied to the plants. Treatments were rotated bi-weekly between four compartments to avoid placement effects, but the control plants remained in the chamber.

We came up with the idea to use liquid ammonia (NH_4OH dilutions). Since the cleansing liquid fully and readily vaporizes at ambient temperatures, the constant re-charge and volatilization of diluted solutions in each of the four greenhouse units should lead to a constant ammonia concentration as long as the ventilation rate stayed appropriate. By knowing the volume and air exchanges of the greenhouse compartments and the mass of volatilized ammonia over time, we were able to adjust the mean NH_3 concentrations in each of the greenhouse units. Calculations were made taking into account the air mass exchanges over time to derive the dilution factors and flow and concentrations of liquid ammonia over time.

We assumed that the evaporation of low quantities of liquid ammonia into a defined air volume would not create phytotoxic peaks and gives a much better control over the mean concentrations than in the experiments of Jones et al. (2013), in which solutions of NH_4OH were filled into plastic beakers and replaced only weekly. The authors knew that this would eventually create potentially phytotoxic ammonia peaks and mentioned that plants were kept at a maximum distance from the saucers.

We developed an ammonia fumigation system that was based on the gentle vaporisation of liquid ammonia into four greenhouse compartments. To fine-tune the dosage and the involved solutions, we had to determine the flux rates (mean air exchange cycles) and ammonia concentrations in each of the five chambers including the control without supply of liquid ammonia. Figure 7 gives an overview of the system.

In the first step, liquid ammonia (Meffert GmbH, Bad KJreuznach) had to be led via a four-channel peristaltic pump (Heidolph Instruments GmbH & CO. KG, Schwabach) to each of the four chambers, where ammonia was added. The pump was equipped with 0.2 mm peristaltic tubes that were supposed to transport the solutions over several meters into each of the greenhouse compartments. After testing the pump with water in our laboratory, it became clear that the normal tube fittings could not be used to connect several tubes without the loss of pressure. We had to thus use only one Santoprene® tube (Spetec GmbH, Erding) per channel, the longest with a length of 20 m and used a cyanoacrylate glue (UHU GmbH & Co. KG Bühl) to fix the snap-grip clamps, which were used to tighten the tubes on the pumping head. We were hence able to lead liquid ammonia into each of the chambers with constant dripping of the liquid at the end of the tube when the pump was active. We had to guide the peristaltic pharmed tubes (0.2 mm inner diameter) through rigid Teflon lines (0.5 cm inner diameter) to avoid bending of the rubberlike material. The Teflon lines were directed via existing ventilation ducts in order to not constitute an obstruction to existing equipment. A maximum distance of 20 m and a height gradient of 4 meters had to be overcome to the most distant chamber, but the peristalsis still worked fine to efficiently transport the liquid ammonia through the tubes.

The peristaltic pump was initially adjusted to a flow of 0.2 ml per minute and a volume output of 0.7 ml per cycle. In order to not have a constant dripping of liquid onto the sponge cloth in each of the chambers, a break of 10 minutes has been programmed in the pump menu being followed by another pumping cycle of 3.5 minutes. A fan helped to vaporize the liquid and the large ventilator at the roof mixed the gaseous ammonia into the chamber air. The pump and the tubes worked without failure during the five months of the fumigation study.

Since real time monitors for ammonia were not available during the project, the realized concentrations were determined on a weekly basis with Radiello® passive samplers (Sigma Aldrich) placed 50 cm above the canopy in each of the greenhouse chambers. The radial diffusive samplers are certified according to EN 17346 and have a high adsorption efficiency for ammonium. The method is based on the protocol by FSM (2020) involving the indophenol method. Because temperatures did not fall below 20°C throughout the experiment, results did not have to undergo a temperature correction.

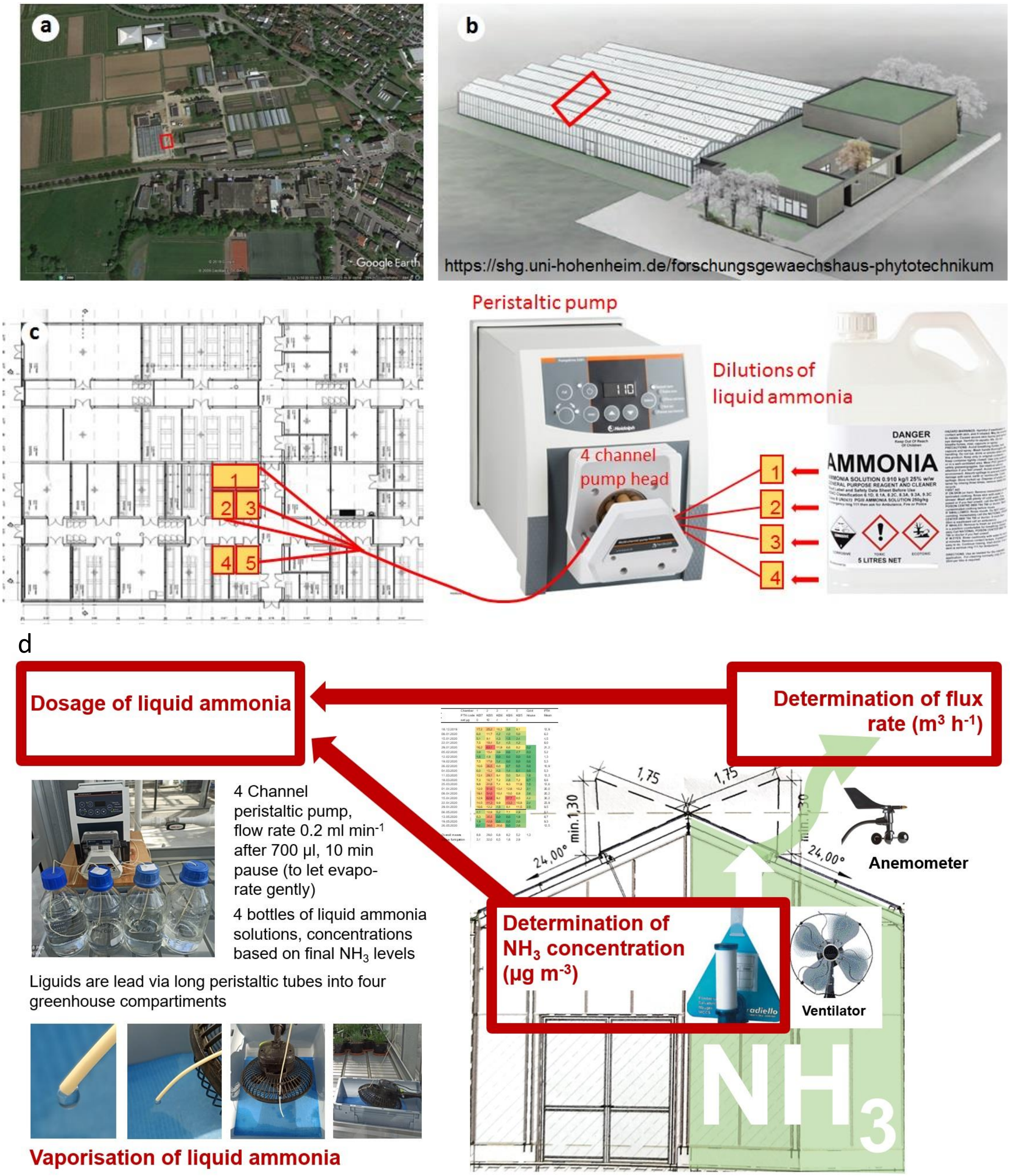
On 6 of May 2020, we began with the supply of liquid ammonia (a ten-fold dilution) to one of the chambers and iteratively tuned in the concentration by reducing the flow of the peristaltic pump and by further diluting the ammonia in the stock solution in the following weeks. On 19 May 2020, we proceeded with the second highest concentration by using half of the dilution of liquid ammonia used for the highest level. After measuring the realized NH_3 concentrations and adapting the dilutions another time, we finally came up with following NH_4-OH concentrations used after the mid of June 2020 until the end of the fumigation:

- 20 ml of NH_4OH (9.5% solution) made up to 1 L (1:50) for the highest concentration
- 10 ml NH_4OH (9.5% solution) made up to 1 L (1:100) for the second highest
- 5 ml NH_4OH (9.5% solution) made up to 1 L (1:200) for the third highest
- pure water for the lowest concentration. The control was not supplied with solutions from the peristaltic pump.

The flow of the peristaltic pump was adjusted to 0.2 ml min^{-1} for 3.5 minutes followed by a break of 10 minutes. By this, 75 ml day^{-1} of the four solutions were added to four chambers.

Figure 7: Overview of the ammonia exposure system established in four chambers of the PHT

Location of the PHT to the west of the University of Hohenheim (a) and position of the chambers involved for the fumigation experiment (red boxes in b, c). Underneath (d), a general scheme is shown giving an overview of the methods used for the fumigation.



Source: Own illustration using own photographs, google maps and information from the PR Department, University of Hohenheim.

3.2.2 Results

3.2.2.1 Ammonia concentrations and temperatures

In the following paragraphs, we will give details on the methodology, quality checks and modifications of the ammonia measurements. We used Radiello® passive samplers for the determination of ammonia concentrations in five greenhouse compartments and exchanged the adsorption cartridges once a week to have a satisfactory resolution of ammonia levels over time.

During the first seven months of the experiments (WP2), the samplers were analyzed in the lab of the Institute of Landscape and Plant Ecology. Aqueous extracts (10 ml) of the cartridges were pipetted manually into test tubes to which various chemicals had to be added. The methodology follows the protocol specified in FSM (2020). At first, 10 ml of deionized water were added to the tube and the sample was stirred for at least 10 seconds to release the adsorbed ammonium into solution. 1 ml of this solution was transferred into a test tube along with 0.4 ml of phenol solution, consisting of 10 g of phenol in 100 ml of ethanol. Furthermore, 0.4 ml of cyanoferrate solution was pipetted into the tube, consisting of 0.5 g sodium pentacyanonitrosylferrate dihydrate ($Na_2Fe(CN)_5NO \cdot 2\ H2O$) dissolved in 100 ml of water plus a few drops of 10 % NaOH. Finally, 5 ml of buffer solution were added to the tube, consisting of 1.1 g NaOH and 3.04 g of $NaHCO_3$ followed by 1 ml of oxidizing solution, consisting of sodium hypochlorite with 1% of active chlorine in 0.2 M NaOH.

The final mixture was allowed to react for at least one hour to yield the blue colorant indophenol and the absorbance of the solution was measured at 635 nm using a spectrophotometer. Deionized water was used to zero the spectrophotometer and NH_4^+ calibration curves were prepared every day when samples were analysed using ammonium chloride standards in the range from 0.5 to 50 mg l^{-1} to calibrate the analyses. The absorbance of the samples and the ammonium standards were then entered into an Excel® sheet along with the time of exposure and a fixed adsorption coefficient to calculate the mean ammonia concentrations. The determination of the ammonium ion was based on spectrophotometric analyses and made use of the blue indophenol colorant.

In order to check the quality of our analyses, we handed extracts of 20 passive samplers, in which we had determined NH_4^+ before, to the Core Facility Hohenheim (CFH) Module 3 Analytical Chemistry. We wanted to let them analyze ammonium in the same extracts to test the validity of our previous measurements. The samples stemmed from the four weekly sampling campaigns from 23 June to 14 July 2020.

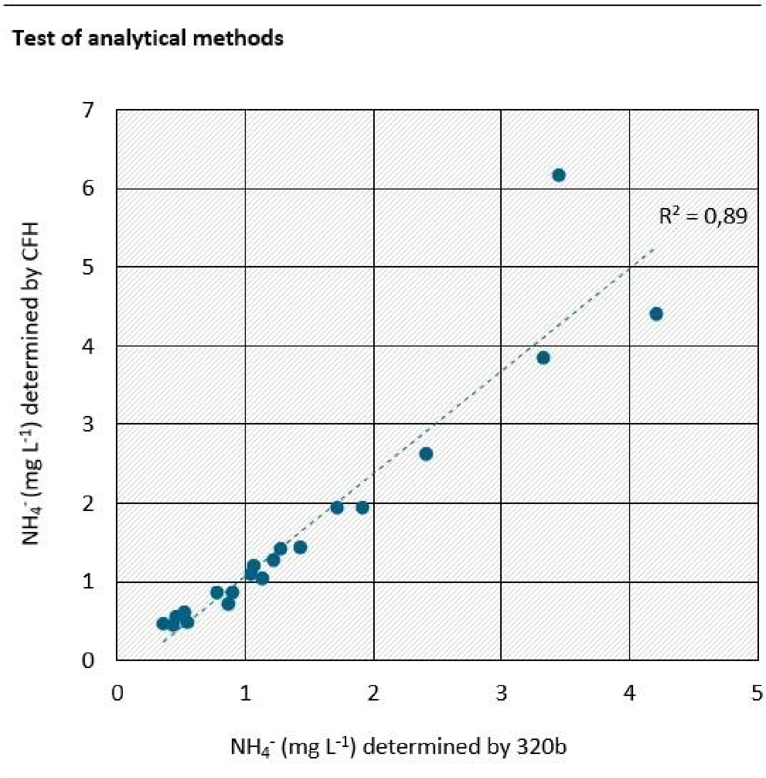
A continuous flow analyses (CFA) system, Evolution II (Alliance Systems GmbH, Freilassing) is being used in the CFH for the determination of mineralized soil N (Nmin). The FLOWSYS equipment consists of a sampling unit, a manifold tray to let the sample react with the working reagents and a flow cell detection unit. The automated system is used on a routine basis for agricultural research to determine plant available nitrogen fertilizers in soil extracts and water samples. In the case of ammonium, the measurement principle, like the one used in our own lab, makes use of the indophenol method.

Figure 8 shows that our own measurements agree very well with those from the CFH. The least squares coefficient of determination (R^2) explained most of the variability of the data from one method as related to those measured with the other method. After these laboratory checks, we hence subjected the liquid extracts from the passive samplers to the CFH, which somewhat reduced analytical costs. However, significant costs still arose from purchasing the Radiello® samplers.

Results of the 25 weeks of the fumigation period (almost six months from May to October 2020) are given in Table 7. Mean temperatures for these weeks are presented on the right hand of the table. As can be seen, we were able to create concentration differences between the four treatments (columns), in which ammonia solutions were vaporized into the chamber air. However, the levels fluctuated significantly over time. While in the beginning, concentration differences were large between the treatments, levels became more similar and generally dropped throughout the later summer. It may well be that the higher temperatures in the PHT chamber led to slightly more convection and thus a blow off of ammonia through the roof windows. At the same time, it may be hypothesized that more ammonia was taken up by the growing plants that over time ran into nutrient deficiencies.

It must be noted here that originally, we expected to be able to create a higher air exchange, but the ventilators in the greenhouse compartments did not lead to a high turbulence. Wind speed at the greenhouse roofs were generally very low and could not be determined due to the higher starting torques that would be necessary to start and rotate the anemometer cups. Nevertheless, we were satisfied with the realized ammonium concentrations. Mean ammonia values varied from 4.3 µg m^{-3} in the lowest treatment to 17.6 µg m^{-3} in the highest treatment and are thus well in the range of concentrations to test the validity of critical levels.

Figure 8: Scatterplot showing the good agreement between our own analyses (320b) and the results determined by CFA in the Core Facility Hohenheim (CFH)



Source: Own illustration, University of Hohenheim.

Temperatures were registered continuously using TinyTag Loggers (Gemini Ltd., Chichester), which were placed at a height of 2m in the center of each greenhouse compartment. Loggers were moved with the plants and the ammonia samplers from one chamber to the next at the fortnightly rotation dates. We were thus able to follow the temperatures the plants were exposed to during the complete season and could judge whether the climate was the same for the five ammonia treatments. Table 7 (right hand) shows the mean weekly temperatures. During the fumigation period from May to October 2020, the PHT chambers were about 7 °C warmer on average than outdoors, which would be acceptable for growing heat demanding cultures. For the "cultivation" of wild and mountain plants like *Arnica montana* the climate must nevertheless be seen as highly artificial and rather unsuited. Further comments on plant responses to highly elevated temperatures and pest infestations are given in the following paragraphs and chapters.

Maximum hourly temperatures reached 47°C in the chambers while outdoors, the maximum hourly temperature was 33°C on 31st of July 2020. It must be noted that temperatures were also too high in the cold house, where the plants were kept during the anteceding winter. After the fumigation, we transferred the plants outdoors to a fenced but unroofed place to hibernate the specimens at ambient temperatures during the winter 2020/21. The climatic situation plants were exposed during their whole life cycle is presented in chapter 3.1.3.

Temperatures reached over 40°C in the summer months for several days/weeks and could not be brought down due to the lack of an active cooling system. This caused an extreme evaporative demand in most of the plant species, an increased pest pressure and it furthermore altered the flowering phenology of the plants. Only three out of seven species produced flowers, although all plants were already in their second year of existence and should have proceeded into the generative phase.
There was no opportunity to increase air ventilation, i.e. to remove the heat. The built-in fans in the small compartments were simply not powerful enough to efficiently exchange air during the summer months. Unfortunately, it was not possible to exchange these integrated fans or to equip the compartments with additional air conditioners. The idea to add more powerful fans into the glass panes at the sides of each chamber was rejected by the greenhouse operators.
In general, greenhouse conditions are rather artificial, be it the light, temperature or air movement and turbulence parameters. While typical heat demanding horticultural crops like tomato, peppers and cucumbers can all be grown in greenhouses, wild plant species from temperate regions are very sensitive to extensive heat. Unfortunately, we had to learn this lesson in the summer 2020.

We still acknowledge the benefits of controlled environments (growth chambers) and greenhouses to grow plants under highly standardised conditions with only modifying selected factors (here ammonia). Most of such studies are based, however, on short-term investigations over just a few weeks and do not cover the full life cycle of plants. The extrapolation of results from controlled environments to the field situation will always be critical due to the mentioned artificial conditions in climate chambers and greenhouses. The Service Unit for Greenhouses (SHG) will only in the future establish large walk-in growth cabinets, where long-term trials with a multitude of pots can be performed under controlled conditions. Another fact to mention is that often in closed environments, one will have to face larger pest pressures, which will not occur in the field due to the presence of natural enemies and a better plant hardening due to e.g. the oscillation of daily temperatures.

Table 7: Mean weekly concentrations of ammonia in the five PHT chambers (left) as compared to the mean temperatures (right)

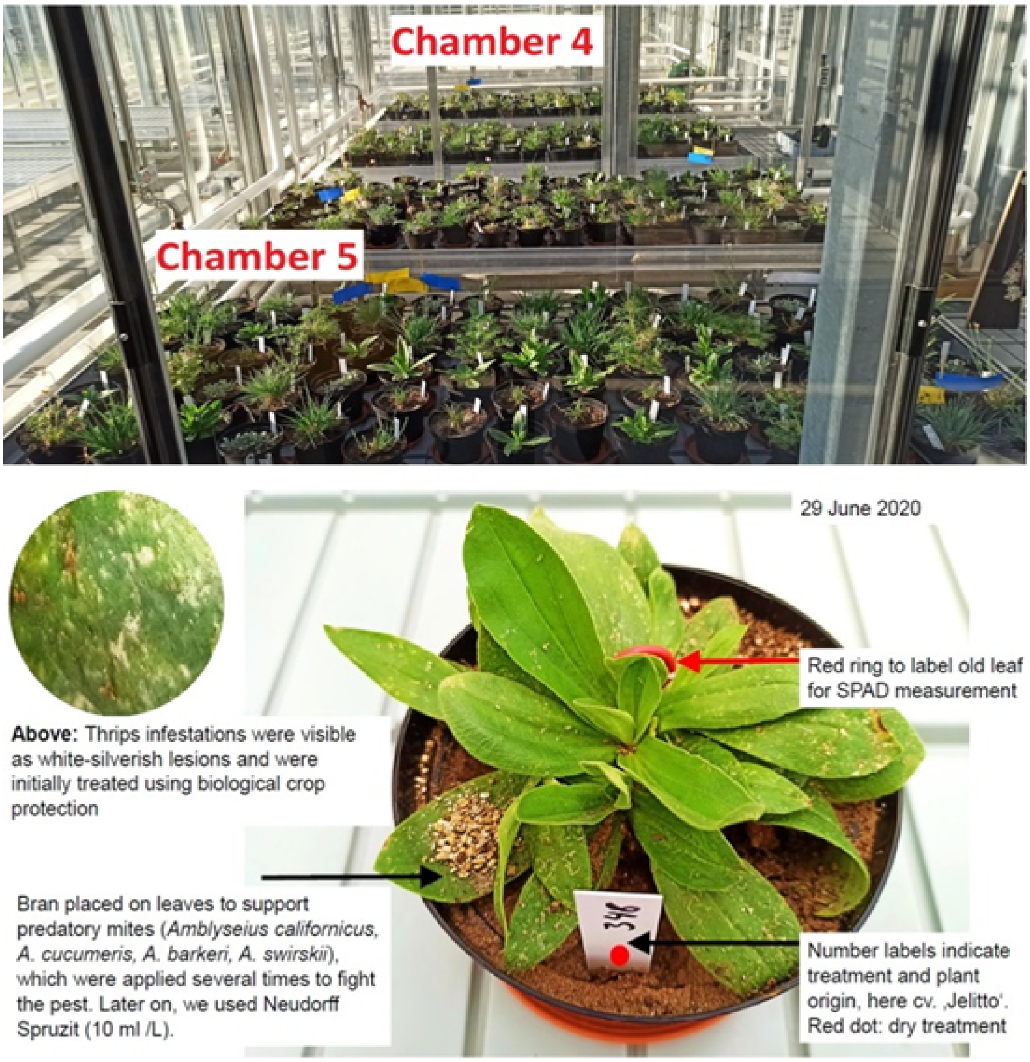
The means over the 25 weeks of the fumigation study are given in the last line. Chamber 1 was used for the control (treatment 1) and remained static, whereas the other treatments were rotated every fortnight between the chambers 2 to 5 later in the season. Numbers 1 to 5 indicate the different ammonia treatments in the upper line, i.e. the set concentrations. "DOY" = day of the year, "DOE" = day of the experiment.

				NH_3 (µg m^{-3})					Temperature (°C)				
	DOY	Week	DOE	1	2	3	4	5	1	2	3	4	5
06.05.2020	127	1	7	4,1	7,1	4,9	5,2	10,9	16,7	17,9	18,4	19,3	18,9
13.05.2020	134	2	14	6,3	0	1,9	0	35,5	20,1	21,1	20,4	21	20,8
19.05.2020	140	3	20	1,9	0	2	0	42,8	19,3	19,8	19,2	19,9	19,8
26.05.2020	147	4	27	0,1	0	2,8	20,6	39	22,2	23,4	22,9	22,5	22,3
02.06.2020	154	5	34	7	10,1	9,4	15,8	14,5	23,3	23,5	23,2	23,2	23,2
09.06.2020	161	6	41	4,7	9,1	12,2	20,3	35,5	21,4	22,3	22	21	21,1
17.06.2020	169	7	49	5,4	6	14,6	20,8	26,7	21,7	22,4	22	21,3	20,9
23.06.2020	175	8	55	3,3	3,1	6,4	11,5	14,5	23,9	24,9	24,6	23,8	23,6
30.06.2020	182	9	62	4,6	4,5	6,6	8,8	17	27	26,8	26,6	27,4	27,7
07.07.2020	189	10	69	2,4	2,2	5,9	7,4	21,7	25,6	25,5	25,2	26,8	26,9
14.07.2020	196	11	76	3,9	1,8	5,4	6,3	17,6	25,5	25,8	25,5	26,5	26,6
21.07.2020	203	12	83	9	4,1	7,9	9,2	40,6	25,5	25,4	25,3	25,8	25,9
28.07.2020	210	13	90	8	10,7	3,2	6	8,8	27,2	27,8	27	27	27,8
04.08.2020	217	14	97	6,8	12	3,5	7	11	28	28,4	27,7	27,6	28,4
11.08.2020	224	15	104	12,9	5,6	5,4	8	12,8	28,7	28,7	28,5	28	27,8
18.08.2020	231	16	111	11,7	5	6,3	8,1	14,2	27,5	27,7	27,6	27,2	27,1
25.08.2020	238	17	118	8	3,9	4,8	1,1	10,3	26,1	25,5	26,2	26	25,4
01.09.2020	245	18	125	3,8	3	3,8	6	9,8	21,7	21,6	22,4	22,2	21,5
08.09.2020	252	19	132	3,3	2,5	4,3	5,1	10,6	22,1	21,9	21,4	22,1	22
15.09.2020	259	20	139	6	3,2	5,9	6,4	11,8	24,6	24,3	24,1	24,5	24,3
22.09.2020	266	21	146	7,5	3,9	5,8	8,1	9,8	23,3	23,1	23,1	23,1	23,1
29.09.2020	273	22	153	3	2,3	3,2	7,8	8	17,6	18,3	17,6	17,7	18,5
06.10.2020	280	23	160	2,2	3,1	5	7,5	7,6	16,7	17,3	17	17	17,6
13.10.2020	287	24	167	1,6	2,1	3,3	6,7	6,8	14,9	15,7	15,2	15,2	16
20.10.2020	294	25	174	1,7	1,8	2,5	5	3,2					
mean				**5,16**	**4,29**	**5,48**	**8,35**	**17,6**	**22,9**	**23,3**	**23**	**23,2**	**23,2**

3.2.2.2 Plant responses

After the transfer of all pots to the greenhouse, the plant species developed differently. Whereas some species grew well and entered the flowering stage and/or produced seeds, other species did not change much in growth and did not flower although it was the second year of their cultivation. Figure 9 gives an overview of plants in two fumigation chambers. Species also differed with regard to pest infestations and their reaction to the extreme heat in the greenhouse. *Arnica montana* (both origins) was the only species that had to cope with severe thrips infestation and was treated several times, accordingly. The other species did not show any pests and all species developed well without the supply of fertilizers.

Figure 9: Overview of plants in two chambers of the PHT (above) and a plant of *Arnica montana* infested with thrips (round insert)



Source: Own photographs and illustrations, University of Hohenheim.

Water supply

Watering the plants was very time-consuming during the summer because all pots had to be watered individually and the water amount need to be noted. This took two to three hours on average when all plants needed to be watered. During the hot summer days, when temperatures reached over 40°C in the greenhouse, watering needed to be done every day. Water used for the plant watering stemmed from a large cistern, which collects the rainfall reaching the roofs of the PHT. In order to address how much nitrogen would have been introduced into the pots with the manual irrigation, water samples were analysed for nitrate and ammonium at the end of the growing period. All five replicates showed values below 0.2 mg l^{-1}, for ammonium and nitrate, respectively. We can thus be sure that only a low amount of additional nutrients was added to the pots via watering and that a potential growth stimulation via the supply of water would be negligible. Based on the water volumes, the plants received until the intermediate harvest (IMH) and the end of the growing season (10 October 2020) and their total dry biomass, we calculated the water use efficiency (WUE) as an additional parameter. We expected that the species would show large differences in WUE due to their different ecological behaviour and adaptation to dry or moist environments. We wanted to see whether ammonia or the availability of nitrogen would affect the way how plants dealt with the resource water.

Half of the plants were grown in "dry treatments" (DT) and we aimed to supply around 33 % less to this set of plants as compared to the "well-watered" (WW) plants. However, this was sometimes difficult, especially during the very hot days when all plants needed a lot of water in order not to die. We were able to reduce watering for about a third for almost all species, both until the intermediate harvest (IMH) and until the end of the greenhouse season. Table 8 shows how these water supplies compare to the long-term precipitation data in Hohenheim. We based our evaluation on the 30-year climate normal period (CNP) from 1981 to 2010.

As previously described, half of the plants were watered according to their needs (Well-watered, WW) and the other half of the plants should receive 33 % less water (Drought treated, DT) in order to simulate a drought stress representing a growth period comparable to the summer 2003. For each individual pot, we noted the watering over the growing period and calculated the means over ten replicates per treatment (Table 8). The water volumes supplied to a pot with an area of 169 cm^2 were recalculated to L m^{-2} or mm.

Table 8: Overview of the water volumes plants received during the fumigation period

Data for Hohenheim represent the 2020 data from LTZ. Long-term records obtained from DWD.

Plant species	Water treat-ment	Total water sum mm pot^{-1} (until 10th October)	Water sum L pot^{-1}	Simulated Rainfall Lm^{-2}	% reduction against moist treatment	% of the rainfall in Hohenheim (CNP 1981-2010)	% of the rainfall in Hohenheim 2020 (against outdoors)
Antennaria dioica	WW	4541	4,5	258	-22,2	-35,8	-18,1
	DT	3524	3,5	200		-50,2	-39,6
Arnica montana (J)	WW	6603	6,6	375	-24,7	-6.7	+13.8
	DT	4971	5,0	282		-29,7	-13,6
Arnica montana (W)	WW	5661	5,7	322	-18,4	-20,0	-4,8
	DT	4621	4,6	263		-34,7	-20,7
Carex arenaria	WW	5776	5,8	328	-28,4	-18,4	-31,8
	DT	4135	4,1	235		-41,6	-55,5
Dianthus deltoides	WW	5360	5,4	305	-28,5	-24,2	-10,7
	DT	3828	3,8	217		-45,9	-36,2
Koeleria glauca	WW	6723	6,7	382	-26,7	-5,0	+12.0
	DT	4929	4,9	280		-30,3	-17,9
Molinia caerulea	WW	6685	6,7	380	-23,8	-5,5	+11.4
	DT	5094	5,1	289		-28,0	-15,1
Pulsatilla vulgaris	WW	7482	7,5	425	-30,3	+5.7	+24.7
	DT	5217	5,2	296		-26,3	-13,1

The realized water sums were compared to the actual rainfall during the summer 2020 and the 30-year climate normal 1981 to 2010. To do so, we used data from the station Stuttgart (Leinfelden) Airport from Deutscher Wetterdienst (DWD, daily data download from www.dwd.de) and compared the different water volumes. While the rainfall in Stuttgart amounted to an average of 401.5 mm in the 30-yr climate normal period, precipitation between 5 May and 10 October 2020 reached only 341 mm in Hohenheim (LTZ data). This represented a reduction against the CNP by 25%. For both time spans, until the intermediate harvest (not shown) and until the 10th of October, when all plants were moved out of the greenhouse, we achieved the desired reduced water supply of around 30% in most species (compare the columns % reduction against the moist treatment in Table 8). In the table, it also becomes clear that the species differed somewhat in their water demand due to the fact that some species grew faster than others. The pre-grown plants of *Pulsatilla vulgaris* e.g. had a greater demand and therefore received more water (in total and when compared to the actual rainfall). The other smaller species had a likewise lower demand and received less water (compare the columns water sum and the % of the rainfall in Hohenheim). In the small plant and drought adapted dry heath species *Carex*, *Dianthus* and *Antennaria*, plant individuals received less water in the moist treatments than would have been available in a season representing a 30-year climate normal.

Individual species accounts

The timing and type of the parameters we studied is summarized in Table 9. In most of the species an intermediate harvest was performed during the season. After the ceasing of the plant

Table 9: Parameters measured over time and chemical composition of the substrates

HEI=Height, FLON=Flower number, SEN=Senescent biomass, LEAFN=Leaf number, SHOOTN=Shoot number. Some parameters were collected before plants were exposed to ammonia (beginning of May 2020).

Species	*Antennaria*	*Arnica*	*Arnica*	*Carex*	*Dianthus*	*Koeleria*	*Molinia*	*Pulsatilla*
HEI2				29.07.20				14.04.20
HEI3								14.05.20
FLON1					22.05.20		25.03.20	25.03.20
FLON2					25.06.20		03.06.20	15.04.20
FLON3							21.07.20	
FLON4							07.09.20	
SEN1		08.07.20	08.07.20		12.08.20	20.07.20	21.07.20	19.05.20
SEN2		30.07.20	30.07.20					30.06.20
SEN3		28.08.20	28.08.20					29.07.20
SEN4		08.10.20	08.10.20					19.08.20
LEAFN1		08.04.20	08.04.20					
LEAFN2		22.04.20	22.04.20					
LEAFN3		12.05.20	12.05.20					
SHOOTN1		22.04.20	22.04.20	03.06.20			08.04.20	
SHOOTN2				29.07.20			03.06.20	
Intermediate Harvest (CUT)	29.07.2020	Continuous harvest of SEN	Continuous harvest of SEN	08.07.20	30.06.20	20.07.20	21.07.20	29.07.20
Final Harvest		14.12.20 (SEN 5)	15.12.20 (SEN5)	11.01.2021 (SEN1)	13.01.2021 (SEN2)	08.12.20 (SEN2)	25.11.20 (SEN2)	26.11.20 (SEN 5)
Substrate								
C(%)	2,7	3,6	3,6	5,7	9,5	8,4	8,4	4,7
N(%)	0,1	0,2	0,2	0,2	0,1	0,2	0,2	0,1
C:N	30,2	22,8	22,8	37,3	122,9	47,2	47,2	50,7
δ15N	44,9	27,8	27,8	75,8	43,1	24,8	24,8	22,4
δ13C	-25,9	-26,4	-26,4	-26,1	-26,1	-25,3	-25,3	-25,5

growth in the late summer, a second harvest, in which the total aboveground biomass was cut again at a height of 5 cm was performed for *Pulsatilla*, *Molinia*, *Koeleria, Dianthus* and *Carex*.

To address subsequent growth in the plants of *Arnica,* we collected the dead (senescent) leaf mass on four dates during the season and prevented to damage the green leaf mass. In contrast, the dead leaf matter of *Antennaria* had already decomposed and could not be collected in October 2020. However, leaf decomposition was only affecting a few leaves and in contrast to the other species, the plants stayed completely green during the winter.

The comparison between the shoot masses of half of the plants that were cut in an intermediate harvest (CUT) in the summer 2020 to the other half that were left intact (UNCUT) until the end of the season (e.g. in *Molinia*), enabled us to also address the effect of mowing, i.e. the removal of nutrients. We expected that an intermediate harvest would overall increase the productivity of some plant species (overyielding) and would also interact with the nitrogen

Table 10: Results of the analyses of variance performed for the plant parameters determined in 2020

Influence of water supply (W-Water) and ammonia (T-treatment) and their interaction (I) on the different parameters were determined via analyses of variance (two-way ANOVA) and multiple comparisons (Tukey Tests) using the free software R with a significance level of p=0.05. The symbol "✓" indicates a significant effect (p<0.05). Note that not all parameters were determined in all of the species at a specific date because we had to respect the different phenology of the species. HEI=Height; FLON=Flower number; SEN=senescent biomass; LEAFN=Leaf number; SHOOTN=Shoot number. Intermediate Harvest (Biomass IMH, cut plants) = Green + senescent biomass until first harvest date (for Arnica = SEN1). Final Harvest = Biomass harvested at second harvest date from CUT and UNCUT plants; TOTAL = Total biomass of the whole season with/without previous harvest, i.e. plants that were subjected to an intermediate harvest or left UNCUT during the season.

	Anten.			***Arnica* (J)**			***Arnica* (W)**			***Carex***			***Dianthus***			***Koeleria***			***Molinia***			***Pulsatilla***		
	T	W	I	T	W	I	T	W	I	T	W	I	T	W	I	T	W	I	T	W	I	T	W	I
HEI1																								
HEI2											✓	✓												
HEI3																								✓
FLON1																								
FLON2														✓										
FLON3																				✓				
FLON4																			✓	✓	✓			
SEN1					✓			✓										✓					✓	
SEN2						✓																		
SEN3					✓			✓	✓															
SEN4					✓	✓																	✓	
LEAFN1																								
LEAFN2																								
LEAFN3																								
SHOOTN1										✓	✓													
SHOOTN2										✓	✓													
Biomass IMH		✓			✓			✓			✓	✓	✓	✓		✓	✓			✓	✓			
Final CUT				✓	✓					✓	✓		✓		✓	✓	✓	✓				✓	✓	
Final UNCUT											✓		✓	✓		✓	✓		✓	✓	✓		✓	
TOTAL CUT				✓	✓			✓		✓	✓	✓		✓		✓	✓			✓	✓		✓	
TOTAL UNCUT											✓			✓		✓	✓		✓	✓	✓			
Water Use Eff.		✓	✓	✓	✓			✓			✓		✓			✓	✓				✓		✓	

loads plants can take up after removing nutrients (reduction of compensation points). A summary of the plant responses and results of the analyses of variance to test the effects of drought and ammonia and their interactions is presented in Table 10.

We analysed the plant data with the free software R and conducted a two-way ANOVA with the factors ammonia concentration and watering. With the help of the command "as.factor", we defined the ammonia concentrations and the water level as factors and conducted the ANOVA for the respective endpoints of interest. With aid of the ANOVA, we were able to see whether there were significant differences between the five ammonia treatments, but sometimes significant differences were found between the lowest and the second or third lowest concentrations. In order to further analyse the ANOVA results, we chose the post-hoc Tukey test that showed where the differences between the treatments could be confirmed. We hoped to find significant differences between the highest and lowest ammonia concentrations, but this was rarely the case.

In Table 10, it can be seen whether the respective treatment had a significant influence on the respective endpoint and in the extensive tables in Annex 3 (p. 170), the outcome of multiple comparisons of means for all the tested species and parameters is given to further evaluate the findings. Results of the Tukey HSD tests have been made separately for each of the four combinations (water supply and cutting) to see if the ammonia treatment caused significant effects. Generally, we found only a few significant effects, which will be elaborated in the following sub-chapters for all the species. The numbers in brackets refer to the original pot number codes that were used for the 100 plant individuals per species resp. plant origin. For a complete list of abbreviations and explanation of all parameters studied it is referred to Annex 2 (p. 168). In the following chapters, however, we will use the full description besides the abbreviation of parameters.

Graphical representations of the results of the growth responses in the fumigation experiment (first year, 2020) and in the field gradient study (second year, 2021) are presented in Annex 4 (Figure 37) using the same scale.

Pulsatilla vulgaris **(1-100)**

The first plants transferred into the PHT in May 2020 were individuals of *Pulsatilla vulgaris* that had been received from a local garden centre as young pre-grown plants. The first relevant parameter for this species was the height measurement on the 14th May 2020 (HEI3), after plants had been exposed to different NH_3 concentrations for approximately two weeks. We have to keep in mind that NH_3 concentrations in the first days/weeks were still high in some chambers and were downregulated afterwards. For HEI3, no statistically significant differences of the ammonia treatments (TREAT) and the water treatments (WAT) could be detected. However, the interaction of both factors was shown to be significant but the following post hoc test (Tukey) did not reveal any further differences. Besides, this early parameter should not be ascribed too much importance.

With regard to the senescent fractions of *Pulsatilla*, only the watering had a significant influence (SEN1 + SEN4). In both cases, plants that received less water and generally produced more senescent biomass.

The total aboveground biomass at the time of the intermediate harvest (TOT1) consisted of the senescent fractions SEN1, SEN2 and SEN3 and of the green fraction HAR1DW. The statistical analysis did not show any significant differences between the treatments for this endpoint.

On 26th November 2020, the final biomass of all plants was harvested. The fraction obtained was originally named “SEN5” and is presented in Table 9 and Table 10 (as Final CUT or Final UNCUT). We had to distinguish between plants that had been subjected to an intermediate harvest (CUT) and plants that were not harvested before (UNCUT). For the final fraction, a watering effect could be seen for both, pre-harvested and uncut plants. An ammonia effect could only be observed for the pre-harvested, i.e. CUT plants. In terms of the water effect, the DT plants produced significantly less biomass than the WW plants. The ammonia effect in the pre-harvested plants was pronounced as follows: The highest NH_3 treatment produced more biomass than the treatments 2, 3 and 4, but did not differ from treatment 1. For mean values and the results of the multiple comparisons, refer to Annex 3 (p. 170). For the graphical representations of the growth in 2020 as compared to 2021, refer to Annex 4 (Figure 37).

The total biomass that was produced over the whole season only showed a watering effect (less biomass when watered less) when subjected to a cut, but no other effects were observed.

Pulsatilla plants produced more biomass when cut at an intermediate harvest only in the moist treatment. Dry plants produced more total biomass when no intermediate harvest was performed.

The last parameter calculated was the water use efficiency (WUE) until the intermediate harvest that was significantly higher in all NH_3 treatments when plants obtained less water.

***Koeleria glauca* (101-200)**

For *Koeleria* plants, only a few parameters were collected, because the plants lagged behind their normal development during the whole season. Since no flowering occurred, we nevertheless decided to submit half of the plants to an intermediate harvest on 20th July 2020. The harvested plant material (CUT) was separated into senescent material (SEN1) and green material (HAR1DW). SEN1 showed neither water nor NH_3 effects, but an interaction could be detected. With regard to the harvested green material, an effect on a lower biomass production was seen when watered less. The total biomass TOT1(SEN1+HAR1DW) combined the described effects, so that a water effect, as well as an interaction effect were observed.

On 8th December 2020, the final harvest took place and all plants were cut at a height of 5 cm (SEN2). For plants, that had faced an intermediate harvest before, a treatment effect, a water effect and an interaction effect could be observed. For the rest of the plants, a treatment and water effect were pronounced. In both cases, plants produced less biomass at a lower water supply.

With regard to the total biomass produced over the whole season, for both harvest-groups (CUT and UNCUT) a significant NH_3 and water effect can be described. Again, DT plants produced less biomass than WW plants. Regarding both parameters, *Koeleria* plants in the control group (ammonia treatment 1), produced more biomass than in the other treatments. *Koeleria* plants of the moist as well as the dry treatment produced less biomass when no intermediate harvest was performed. The readily growing grass showed a positive growth response due to the cut in the summer. The results of all multiple comparisons (Tukey tests) are presented in Annex 3 (p. 170) and show that the highest ammonia treatment did not cause effects significantly different from the other treatments. Only the percentage of senescent leaf mass of total shoot mass (SENPROCENT) in the WW and CUT combinations differed significantly between the lowest and the highest ammonia treatment. For the graphical representations of the growth in 2020 as compared to 2021, refer to Annex 4 (Figure 37).

***Molinia caerulea* (201-300)**

Molinia was included in our experiment in order to investigate a plant species that has frequently been reported to respond positively to eutrophication. The grass developed fast in the warm greenhouse and even produced flowers that were counted regularly. FLON1 was determined before the NH_3 fumigation, but FLON2-FLON4 were observed on the 3rd June 2020, 21st July 2020 and on 7th September, respectively. For FLON3, no significant NH_3 or water effects were seen, but FLON3 showed a response in terms of watering, namely that less-watered plants produced less flowers irrespectively from the NH_3 treatments. The same effect was observed for FLON4, in both cases (when pre-harvested and when uncut).

Another parameter measured on the 3rd June 2020 was the number of stalks (SHOOTN2). This parameter, however, was neither affected by the NH_3 treatment, nor by the watering.

The intermediate harvest was performed on the 21st July 2020 and again the biomass was separated into senescent (SEN1) and green biomass (HAR1DW). The senescent biomass was neither influenced by water nor by NH_3, but the green biomass showed a significant water and interaction effect that was passed on to the parameter TOT1 which consists of SEN1+HAR1DW. The total biomass was significantly reduced when plants received less water.

The final harvest was performed on 25th November 2020 (SEN2). Plants that were subjected to an intermediate harvest did not respond significantly to the water or ammonia treatments. The other half of the plants that was not cut in between, however, showed a significant ammonia, water and interaction effect. Less-watered plants produced generally less biomass than well-watered plants. Furthermore, the highest NH_3 treatment produced the most biomass (when well-watered and subjected to an intermediate cut) in comparison to the other treatments (see Annex 3, p. 170). For the graphical representations of the growth in 2020 as compared to 2021, refer to Annex 4 (Figure 37).

Molinia plants responded - similar to *Koeleria*- with a higher biomass production when plants had been cut before. With regard to the WUE, only an interaction effect of both treatments could be observed. The trend of a higher WUE for the less-watered plants was, however, visible. *Molinia* is as species that can well handle dry and moist conditions. Further comments on the responses of the species to the factors, drought, mowing and removal of resources are presented in chapters 3.4 and 3.5 taking into account the responses of the field gradient study in the second year of the experiments.

***Arnica montana* Jelitto (301-400) and Weiherberg (401-500)**

One of the relevant endpoints for both *Arnica* origins was the leaf number that was counted regularly during the season. It was counted for the first time on 12th May 2020, only a few days after the fumigation start.

Later in the season, we continued to collect senescent plant material SEN1-SEN4 and shed material was collected on the 8th July 2020, 30th July 2020, 28th August 2020 and on the 8th October 2020, respectively. In *Arnica* Jelitto, strong watering effects on SEN1, SEN3 and SEN4 were found. In the cases of SEN1 and SEN4, less-watered plants produced more senescent biomass. At the time of SEN3, however, less-watered plants produced significantly less senescent biomass. This could be explained by the point in time of the measurement. During the hottest months, *Arnica* plants received a lot of water and the difference between the watering treatments was not as pronounced as in other species (see Table 8). An alternative explanation could be that during August, the watering did not differ much between the water treatments, therefore no differences in senescent biomass production were visible. For SEN2 and SEN4, an additional interaction effect was observed.

In *Arnica* Weiherberg plants, a watering effect was pronounced in SEN1 and SEN 3. For both fractions, the trend of a higher senescent biomass production could be confirmed when water supply was reduced.

In both *Arnica* origins, aboveground biomass was not completely harvested, because we hoped that plants would still flower later in the season and we feared that plants would otherwise be affected in their normal phenological behaviour. This is why for *Arnica* there is no total biomass like for the other species. We chose the first harvested senescent biomass SEN1 to represent TOT1 because it had been harvested in the same time frame like TOT1 in the other species.

The final harvest in both Arnica origins referred to the senescent material ("SEN5" or "ARNSEN5") that was gathered on the 14th and 15th December 2020 for the Jelitto and Weiherberg origins, respectively. Whereas Jelitto plants responded significantly towards water and NH_3 treatment, only a water effect was observed in the Weiherberg plants. A significant effect of ammonia on SEN5 was observed only in the Jelitto plants since the plants from the highest ammonia treatment produced significantly more dead biomass than the plants from the lowest treatment (Annex 3, p. 170). More information on the potential effects of loss of resources by senescence, ammonia uptake and survival of plants in the second season is given in chapters 3.4 and 3.5.

With regard to the WUE, that was in this case calculated until SEN1, the same effect as in previous species was observed. The less-watered plants of both origins showed a higher WUE when compared to the well-watered plants indicating a more economic use of resources under a slight drought.

***Antennaria dioica* (601-700)**

Antennaria did not develop much during the fumigation study and stayed in the leaf rosette stage throughout the season. Normally, it is expected that *Antennaria* flowers in the second year. The plant height did not exceed a few centimetres, therefore we cut the plants at a lower height (of about 2cm) at the intermediate harvest on the 29th July 2020. The harvested plant material did not contain senescent material and was thus directly described as TOT1. The biomass showed a significant water effect at that point of time. Less-watered plants produced less biomass throughout all ammonia treatments. A general NH_3 effect could not be observed and there were also no significant differences between treatments with regard to the shoot masses that were cut at the intermediate harvest (Annex 3, p. 170). For the graphical representations of the growth in 2020 as compared to 2021, refer to Annex 4 (Figure 37).

With regard to the WUE until that point of time, a higher WUE could be confirmed for *Antennaria* in those plants that received less water.

In the end of the season in the fumigation study, we could not perform a final harvest for *Antennaria* plants because some leaves had already started to decompose and we did not want to hinder further plant growth in the next season after having cut too much biomass of the winter-green species.

***Carex arenaria* (701-800)**

Carex plants grew fast, but did not produce any flowers in the season, probably due to the unfavourably high temperatures. The endpoints height and shoot number were determined on the 3rd June and 29th July 2020, respectively. On the first date, plants did not show any effect regarding their height. Their shoot number, however, showed several significant responses: A water effect, namely the production of less shoots when watered less. The observed ammonia effect showed that plants of the highest ammonia treatment produced significantly less shoots than all other ammonia treatments. This would hint to problems the species would experience under eutrophication.

The second height measurement (conducted after the intermediate harvest for half of the plants that were not harvested) revealed a water effect. Less-watered plants were generally shorter at that point of time. As for the second shoot measurement, similar effects as described above could be seen. The water effect showed a reduced production of shoots when plants received less water. The ammonia effect showed a lower shoot production for the highest ammonia treatment when compared to all other ammonia treatments, except for treatment 1.

On the 8th of July 2020, half of the plants were harvested, i.e. cut at a height of 5 cm. Harvested plant material was not separated into senescent and green fractions because of the high effort to separate the many thin leaves. For the harvested biomass at the intermediate harvest, a significant water effect could be described. Plants produced less biomass when watered less throughout all NH_3 treatments.

The final harvest was conducted on the 11th January 2021 since before that date, plants were still green. The biomass harvested was named SEN1 because it was the first senescent biomass harvested. SEN1, i.e. the harvested biomass, was significantly lower in the cut plants when watered less. Furthermore, the NH_3 treatments differed significantly from each other. Treatments 1 and 3 differed significantly from treatment 2, i.e. more biomass was produced, but no other differences were observed (see results of multiple comparisons in Annex 3, p. 170). SEN1 in the cut plants only showed a significant watering effect: At the time of the final harvest, less-watered plants produced less biomass over all ammonia treatments.

The total biomass produced over the season, i.e. TOT1+SEN1, showed water and ammonia effects only when cut during the summer (see Annex 3, p. 170). Less-watered plants produced less biomass than well-watered plants and treatments 2 and 5 produced significantly less biomass than treatment 1. For the uncut plants only a water effect was observed. Also, in this case, less-watered plants produced less biomass than WW plants. *Carex* plants did not respond to the intermediate harvest. In the moist WW treatment, the biomass production was the same for cut and uncut plants and in the DT only a slightly higher biomass production of cut plants could be observed. The WUE did not differ between the ammonia treatments but less-watered plants were observed to generally have a higher WUE than well-watered plants also for this species. For the graphical representations of the growth in 2020 as compared to 2021, refer to Annex 4 (Figure 37).

***Dianthus deltoides* (801-900)**

With regard to *Dianthus* – one of the few species that entered into the generative phase – we counted the flower number on 22nd May and 25th June. The first measurement did not reveal any differences between the treatments, but fumigation and different watering had only started shortly before. In June, the flower number was significantly influenced by the watering and plants receiving less water produced a lower number of flowers per plant. This finding agrees with the reduced flower number in *Molinia* when watered less (see above).

A few days later, on 30th June 2020, half of the plants were harvested. *Dianthus* was the first species to be harvested because the development had already more progressed than in the other species. The material from the intermediate harvest was not separated into green and senescent material because senescent material had not yet been produced. The biomass of the intermediate harvest showed a significant watering and ammonia effect. The known pattern that less-watered plants produced less biomass was seen accordingly. The ammonia effect was recorded in the difference of treatment 2 from treatments 3 and 5, the latter biomass being significantly lower (Annex 3, p. 170).

A few weeks later on 12th August 2020, senescent plant material was collected from the uncut plants. However, no statistically significant differences between the treatments could be seen in the cut plants. On the 13th January 2021 we finally conducted the ultimate harvest of *Dianthus*, where all plants were cut at a height of 5 cm. The harvested biomass was titled SEN2 and did only show an ammonia effect between treatment 3 and 1. Treatment 3 had a lower biomass production than treatment 1. Summing up the biomass from the cut and uncut plants (SHOOT2020) significant differences between ammonia treatments were only seen in the CUT plants. Treatment 2 had significantly higher biomasses than plants from the treatments 3 and 5. All in all, no clear trend could be detected in this highly variable plant species. The other half of the plants – those that had not been harvested before – showed the same response with regard to a lower watering like most other species before: a lower biomass production throughout all NH_3 treatments.

The total biomass produced by plants in the fumigation experiment only showed a water effect. The drought treated plants produced less biomass over the whole season when compared to the well-watered plants. When it comes to the total biomass production of cut and uncut *Dianthus* plants, it can be seen that uncut plants produced only slightly more biomass than cut plants. Regarding the WUE, we could detect an ammonia effect only. Like with the shoot mass, plants of treatment 2 showed the highest values and differed significantly from treatments 3 and 5.

3.3 The field gradient study (2021)

After the overwintering of the 900 plants used for the fumigation study outside of the greenhouse, we originally intended to relocate the pots to the PHT in spring 2021 and planned to subject them to a repeated ammonia fumigation in the same five small compartments, where they had been exposed over the first season. Several disadvantages and shortcomings, however, made us reconsider, adapt and modify these plans. The biggest problem was the heat in the fumigation chambers, which had a larger effect on the plants than the different ammonia concentrations in the chambers (see chapter 3.2.2.1). The high water pressure deficits (VPD) due to heat reduced the gas exchange of plants because of the closed plant stomata, preventing the uptake of CO_2 as well as the influx of ammonia.

We therefore came up with the idea to perform a field gradient study instead to continue in the greenhouses where the summer temperatures were far too high to grow wild plant species adapted to the cool central European climate. We planned to work at the University´s research farm Unterer Lindenhof (ULI) and elaborated a work plan to expose our plants at five locations in the lee of the farm (pig stables) that would be representative for the levels specified in the initial project call. We aimed to expose plants at these locations in spring 2021 and in the winter proceeded to determine ammonia concentrations on a weekly basis.

3.3.1 Experimental design

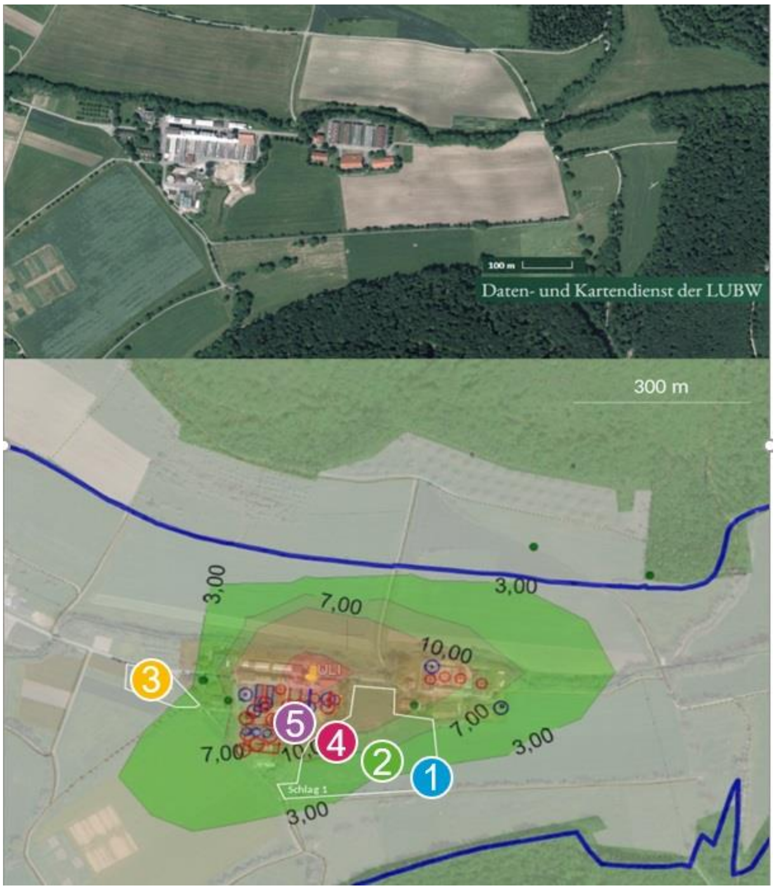
The chosen field site ULI is an experimental farm with livestock and biogas production managed by the Station for Agricultural Sciences of the University of Hohenheim. The research focus is the improvement of agricultural methods in plant and animal production with regard to product quality and sustainability. ULI is located 480 m a.s.l. and 35 km south of Hohenheim in Eningen unter Achalm, near the city of Reutlingen (Figure 10). The selected stations for plant exposure were chosen according to their distance to the pig stable and the biogas plant. We planned to include the existing LTZ weather station at ULI (Figure 11, station 3) because of the direct comparability and possible usage of meteorological data. At first, we planned to use this station as a control station with the lowest ammonia concentrations, but after first measurements it became clear that the control should be positioned to the east, farthest away from the stable (Figure 11, station 1). The figure furthermore shows an aerial view of the farm (above) and modelled ammonia concentrations (µg m^{-3}) after Herdt (2016) as represented by the numbers in the green area (below). The five stations are labelled in different colours, to differentiate between the five treatments. The lowest NH_3 concentration is indicated in blue, the next higher concentration in green, followed by yellow, pink and purple, latter colour representing the highest concentration. After March 2021, each station was equipped with two metal tables (Knecht GmbH, Metzingen). In the end of May 2021, everything was installed and all plants had been moved to the research sites. At each station, we furthermore installed a tall wooden pile where we mounted the Radiello® samplers at 1.5 m above ground, the temperature loggers and a bulk sampler for the collection of rain water. A second wooden post was used at each station and wind sensors were installed at the same height.

Figure 10: Location of the study area "Unterer Lindenhof"

Source: google earth, yellow box indicates study area at ULI (Unterer Lindenhof University of Hohenheim).

Figure 11: Aerial view on „Unterer Lindenhof "(ULI, above) and the chosen stations (below)

The figure below shows the borders to the Natura 2000 site "Mittlere Schwäbische Alb" (blue line) and modelled ammonia concentrations. The numbers 1-5 indicate the location of the stations with increasing ammonia concentrations. Station 3 is located near an LTZ weather station (https://www.wetter-bw.de/Agrarmeteorologie-BW/Wetterdaten/Stationskarte; search "Unterer Lindenhof"). The sublayer of the lower graph shows the results of an ammonia dispersion model performed by Herdt (2016), indicating an ammonia concentration gradient from 3.00 to 10.00 µg m^{-3}.



Source: from Herdt (2016), modified.

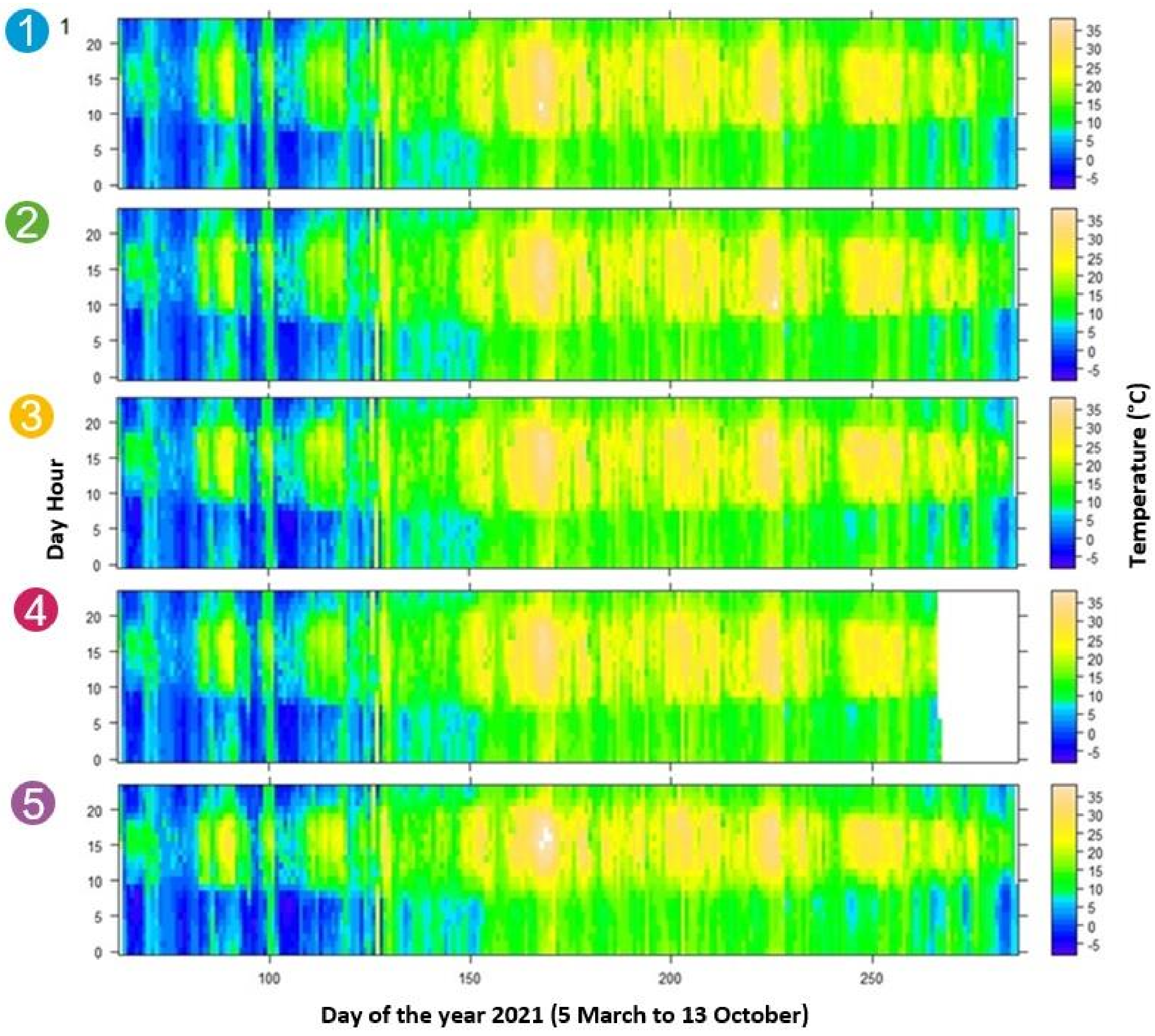
3.3.2 Results

3.3.2.1 Climate and ammonia concentrations in the field (2021)

Heat maps representing the complete 30 weeks of the field gradient study are given in Figure 12, showing relatively cool conditions in March and April 2021 and three shorter warmer spells throughout the summer. While despite the altitudinal gradient of approximately 40 m, mean temperatures at the stations 1 to 3 were identical (14.8°C) until 24 September, slightly lower temperatures were recorded at the locations 4 (14.6°C) and 5 (14.3°C). We assume that this small gradient of up to a maximum of 0.5°C on average, close to the farm did probably not decrease or increase the growth of plants exposed there. Overall, the vegetation period 2021 was rather a cool season as compared to previous and later years, so that growth conditions in the second year of the experiment should have been suitable for all the temperate species.

Figure 12: Temperatures (hourly values) recorded at the five stations at ULI

Temperatures are given as heat maps after the day of year (x-axes) and the time of the day (y-axes). Note that the logger from station 4 failed during the last three weeks. Data were determined as hourly averages. Station codes are represented by numbers and the colours used throughout the report.



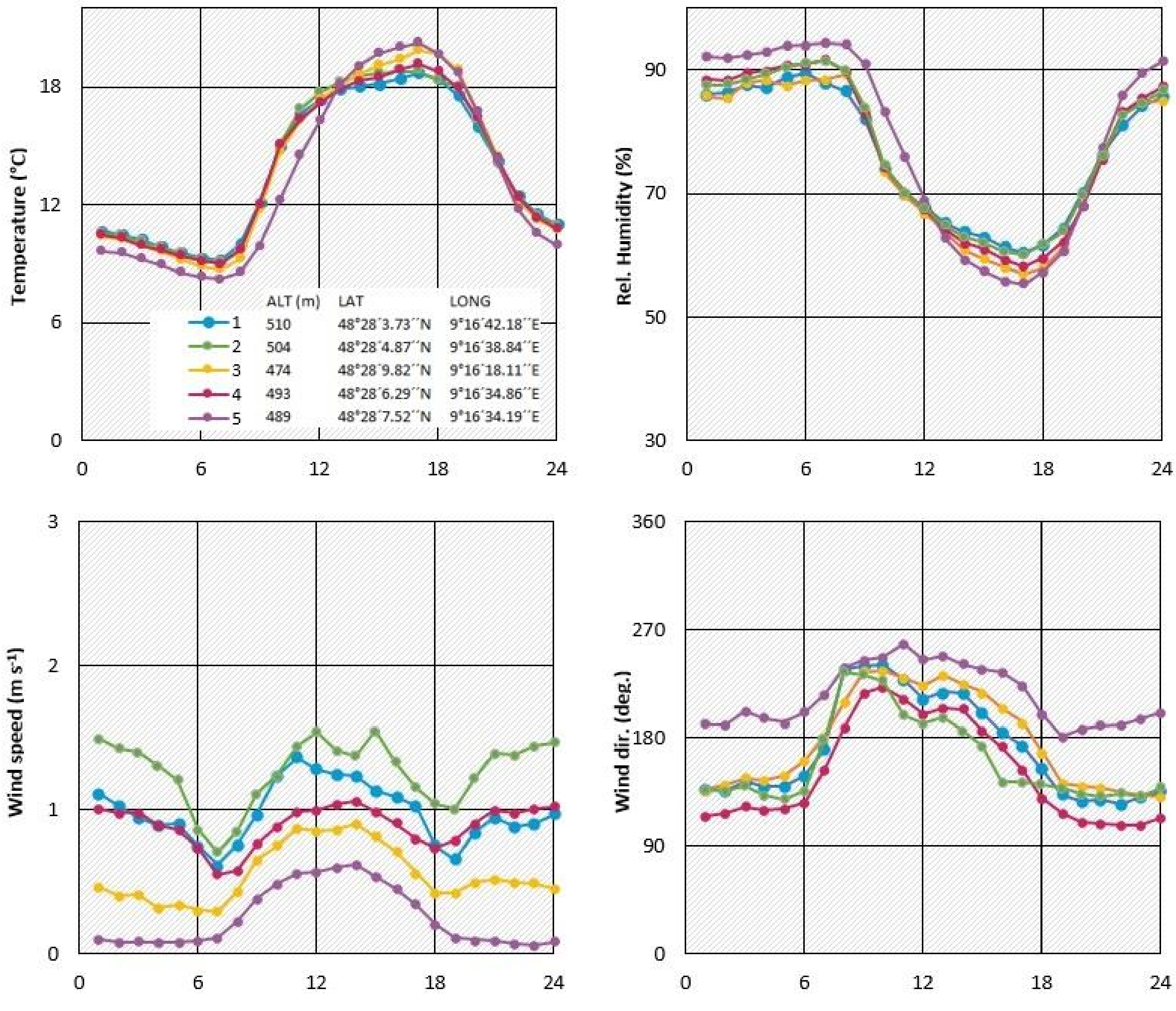
Source: Own illustration, based on heat maps generated with the free statistical software R, University of Hohenheim.

When looking at the heat maps, we can observe that highest noon temperatures were registered at station 5 (white dots representing 35°C at 19/20 June), despite the lower overall average. Interestingly, we can observe that the daily temperature fluctuation at this station differed slightly from the others. The temperature and also the diurnal humidity profiles were somewhat steeper close to the farm, probably due to the station's position in a slight depression behind an embankment.

During the night and the early morning, temperatures were lower and relative humidity higher, whereas conditions in the noon were slightly higher and drier (Figure 13). Latter may be due to the proximity of buildings and the higher cover of sealed surface, which heat up more strongly than the grasslands, where the other stations were set up. A slightly different microclimate at station 5 as compared to the other stations can also be confirmed by the rainfall and wind analyses (see below). For a representation of weekly temperature means and the ammonia measurements, refer to Table 12.

Figure 13: Diurnal profiles of temperatures, relative humidity, wind speed and wind direction recorded at the five stations

Left top: temperatures; Right top: Relative humidity; Left bottom: Wind speed; Right bottom: Wind direction; Insert in panel top left depicts the station numbers, colour codes and their geographical information (altitude and coordinates).



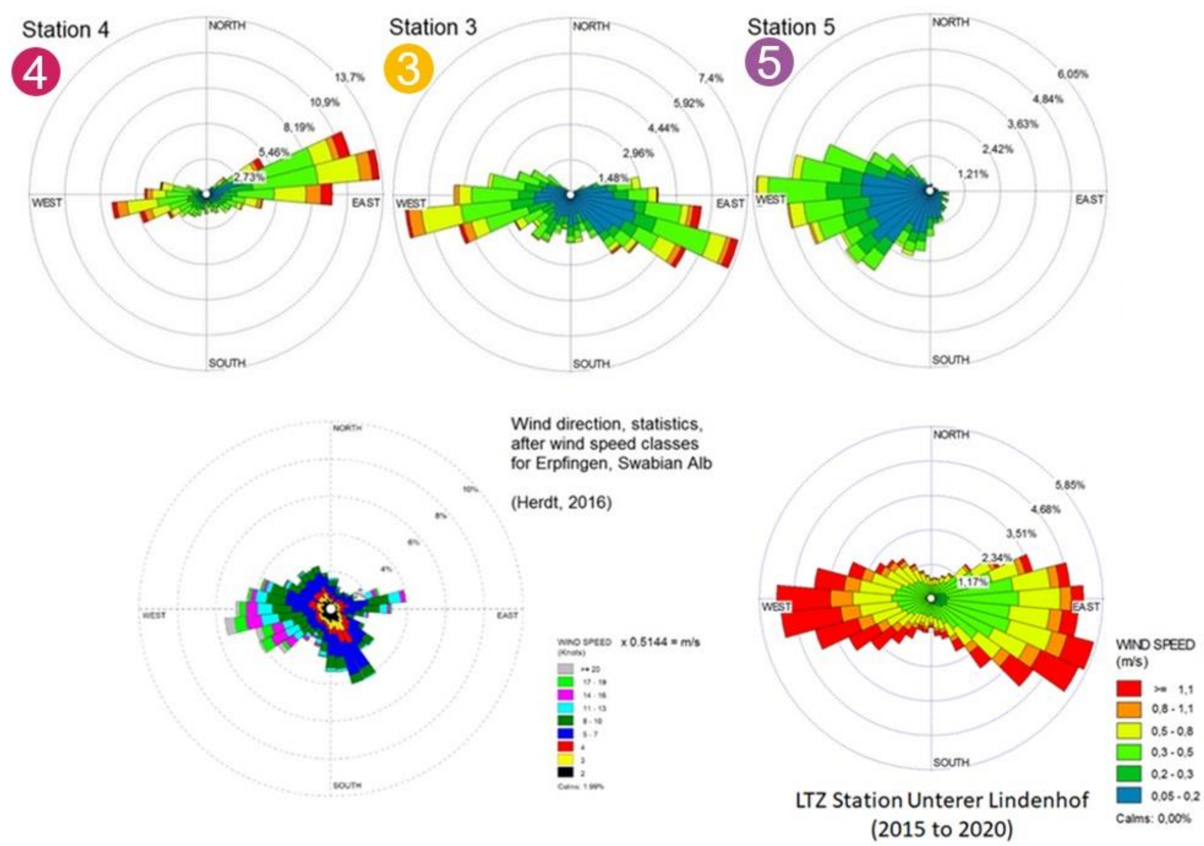
Source: Own illustration, based own climate measurements, University of Hohenheim.

Wind direction and wind speed in 2021

Figure 14 (bottom) shows daily profiles for wind speed and mean wind directions. The data are in line with the temperature measurements and confirm that heat exchange and turbulence are also interconnected at the mesoscale. While during the night wind speed and temperature go down, air exchange in the early morning is driven by the mountain breeze from the “Albtrauf” slope as indicated by the prevailing south-easterly winds. With the heating and breaking of the boundary layer in the morning hours, wind speed goes up again and the mean wind direction moves to a westerly position. However, at station 5 the wind system behaved slightly different with generally lower wind speeds and westerly winds dominating (Figure 14).

Figure 14: Wind roses of stations 3 to 5 at Unterer Lindenhof

The three wind roses from stations 3 to 5 were chosen because their data was available from June to October 2021. Bars indicate the direction (32 sectors) where the wind was blowing from and colours give the frequency of wind speed classes. Bottom left: Wind rose for the climate station Erpfingen that was used in the dispersion model by Herdt (2016) and below right: wind rose for the period 2015 to 2020 using data from the LTZ climate station (https://www.wetter-bw.de/Agrarmeteorologie-BW/Wetterdaten/Stationskarte; search “Unterer Lindenhof”) operated in the west of ULI (identical to station 3 in present study).



Source: Windrose bottom left from Herdt (2016). Other wind roses are own illustrations, University of Hohenheim.

Rainfall and N-deposition in 2021

During the field study lasting from 25 March to 13 October 2021, a precipitation sum of 556 mm was recorded at the LTZ Weather station (https://www.wetter-bw.de/Agrarmeteorologie-BW/Wetterdaten/Stationskarte; search “Uterer Lindenhof”), but own measurements at the same location (Station 3) indicated much higher values of 702 mm. Precipitation sums were even higher at the other stations and highest at station 5, probably due to channelling effects by the nearby buildings. However, wind speed at this station was the lowest. We suppose that the difference in precipitation sums between the five stations was negligible and probably did not create differences in plant growth.

Overall, the season was rather rainy. Dry conditions were faced only in the beginning of June, in the end of July and in September 2021. In these weeks, we had to supply water only a few times, representing volumes of 910 ml for the moist perennial and annual species and 650 ml for the dry perennials, respectively.

Information on the water supply is given in the table below (Table 11). Overall, in 2021 the plants in the "dry treatment" (DT) only received 2.1 % less water than those in the "well-watered treatments" (WW). Compared to the year 2020, we thus did not realize a drought in 2021, but we could still observe some small carry-over effects from the previous year (see results chapter 3.2).

Table 11: Water supply to the perennial plants in the years 2020 and 2021

Data refer to the complete season in 2021 and to the species-specific water supply in the season 2020. Mean water supply in the DT and WW treatments is related to the long-term climate normal for the period 1981-2010 (DWD, Stuttgart).

	Antennaria dioica	*Arnica montana* (J)	*Arnica montana* (W)	*Carex arenaria*	*Dianthus deltoides*	*Koeleria glauca*	*Molinia caerulea*	*Pulsatilla vulgaris*		Mean (L)	Precipitation (mm)	CNP 1981-2010 (mm)	% actual / CNP
2020 (greenhouse)													
Water supply to moist plants (l)	4,5	6,6	5,7	5,8	5,4	6,7	6,7	7,5		6,1	396	395	+0,3
Water supply to dry plants (l)	3,5	5	4,6	4,1	3,8	4,9	5,1	5,2		4,5	295	395	-25
% reduction in dry plants	22	25	18	28	29	27	24	30					
2021 (field gradient study)	Stations												
	1	**2**	**3**	**4**	**5**								
L m^{-2} (via rainfall)	757	761	712	723	811								
L pot^{-1}	12	12	11	11	13								
mL day^{-1}	57	57	54	55	61								
Water supply to moist plants (L)	0,9	0,9	0,9	0,9	0,9								
Water supply to dry plants (L)	0,7	0,7	0,7	0,7	0,7								
Total water supply moist (L)	13	13	12	12	13					13	812	467	+73,8
Total water supply dry (L)	12	12	12	12	13					12	795	467	+70,2
% reduction in dry plants	2,1	2,1	2,2	2,2	1,9								

Greenhouse: 5 May to 10 October 2020 (160 days), DOY 125-183: 395 mm
Field study: 25 March to 13 October 2021 (204 days), DOY 84-284: 467 mm

For a representation of weekly precipitation sums, temperature means and the ammonia measurements it is referred to Table 12.

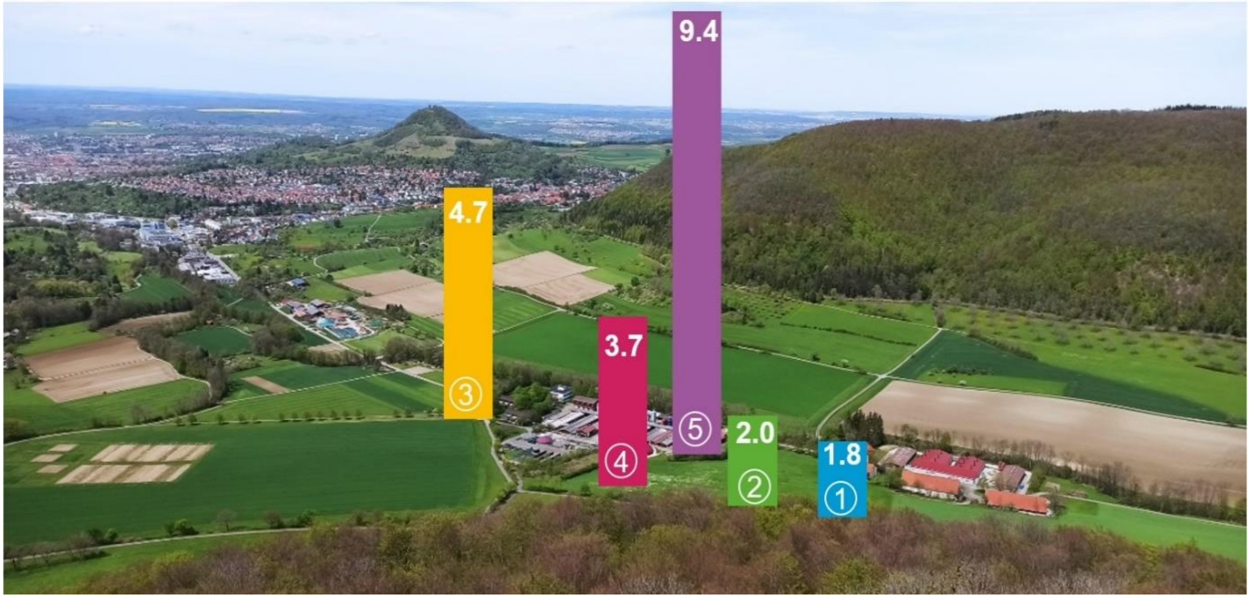
Considering the precipitation sums and the ammonium and nitrate concentrations in the collected water, we were able to calculate the bulk deposition of nitrogen over the growing season. Data are presented in Table 12. Since in the first weeks, we often encountered bird droppings in the funnels we fitted nets around the bulk samplers in the mid of May. The sum of deposited nitrogen between mid of May and mid of October (5 months) was approximated 3 kg N per ha and did not show differences between the five locations. Recalculated to a complete year, the N deposition level would be around 7.2 kg N per ha and year, which is in the range of critical loads for N-sensitive acidic grassland habitats, but would probably not be critical to the calcareous grasslands in the surroundings.

Ammonia concentrations

The results of the weekly monitoring of ammonia using Radiello® passive samplers are also represented in the following table, in which temperature, rainfall as well as bulk N-deposition are all expressed on a weekly aggregation level. Figure 15 shows the mean seasonal ammonia concentrations (bars for the five stations). As expected, highest ammonia levels were recorded in direct vicinity to the stables at station 5 (9.4 µg m^{-3}), while concentrations at station 1 dropped to a mean level of 1.8 µg m^{-3}. Ammonia concentrations were not related to mean temperatures or precipitation, but were mainly affected by the emissions from the ULI farm. This is indicated by the fact that data for station 1 are positively interrelated with those from station 5 (R^2 0.5). Highest agreement in the data was observed between station 1 and station 2 (R^2 0.91), which also confirms that the measurements worked fine. During the first two months of the field study, two samplers were exposed in parallel to check the validity of our measurements. Coefficients of variation were acceptable with values lower than 5%.

Interestingly, very low ammonia concentration and bulk deposition values were determined at the downwind stations between May and July 2021 (see Table 12). We suggest that this may have been caused by the strong growth of the grassland and the "readiness" of the vegetation to absorb ammonia. The underlying mechanism is the growth dilution in the tall grass canopy and the lowering of the ammonia compensation point, leading to an increased flux into the plants. In June 2021, the situation changed probably due to the first cut (mowing) of the grassland on site and the taking off of the vegetation in the neighbourhood. To verify changes in bi-directional fluxes, Eddy Covariance measurements would have to be performed (e.g. Pleim et al. 2019). Weekly ammonia concentrations were averaged for the exposure times of each species in order to calculate dose-response relationships between the ammonia exposure and the specific plant response parameters. These calculated doses are presented in the individual sub-chapters for each of the 13 species.

Figure 15: Seasonal mean ammonia concentrations (µg m^{-3}) at ULI. The bars show mean concentrations over the whole season (18 March-13 October 2021).



Source: Own photograph and illustration of ammonia concentrations as bars, University of Hohenheim.

Table 12: Concentrations of ammonia, nitrogen bulk deposition, temperatures and rainfall at the five stations at ULI 2021

Data are derived from 30 weeks (WK) of the field gradient study. Empty cells refer to data not available. “DOY” day of the year, “DOE” day of the experiment.

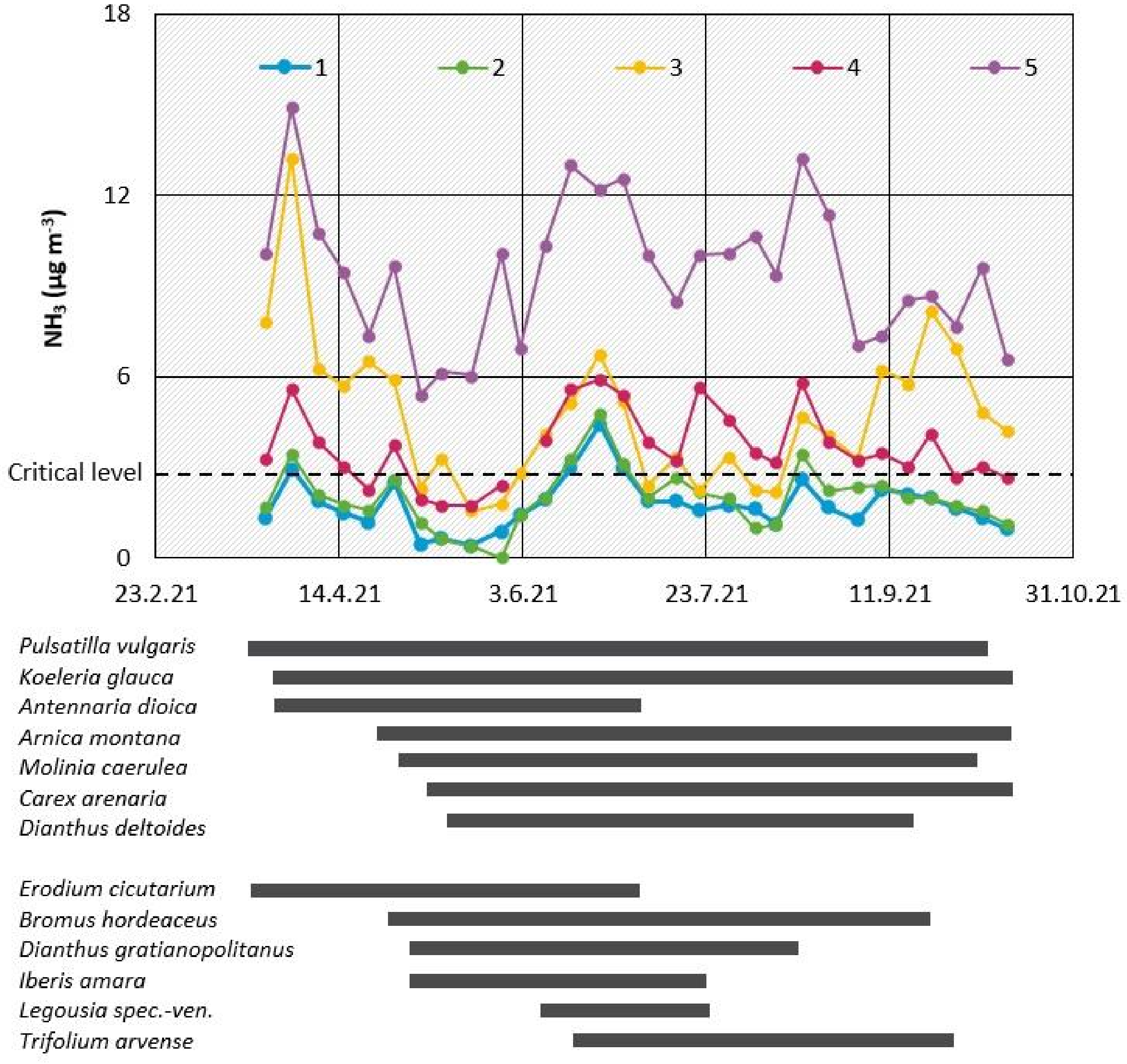
				NH_3 (µg m^{-3})					N-deposition (kg N ha^{-1} $week^{-1}$)					Temperature (°C)					Rainfall (mm)				
	DOY	**WK**	**DOE**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
25.03.2021	**84**	**1**	**7**	1,3	1,7	7,8	3,3	10,1	0,1	0,8	0,1	0,6	0,8	2,9	3,1	3,0	2,9	2,9	1,9	1,3	2,5	2,5	3,2
01.04.2021	**91**	**2**	**14**	2,9	3,4	13,2	5,6	14,9	0,3	0,5	0,2	0,2	0,3	12,0	12,1	11,6	11,3	11,1	8,9	12,7	10,2	9,5	9,5
08.04.2021	**98**	**3**	**21**	1,9	2,1	6,2	3,8	10,8	0,1	0,1	0,1	3,1	0,8	3,8	4,0	3,9	3,7	3,6	5,1	5,7	7,0	8,3	10,2
15.04.2021	**105**	**4**	**28**	1,5	1,8	5,7	3,1	9,5	0,6	0,1	0,2	8,3	1,9	5,6	5,8	5,7	5,4	5,3	19,1	19,1	22,3	18,5	31,2
22.04.2021	**112**	**5**	**35**	1,2	1,6	6,5	2,3	7,4	0,2	0,9	0,1	0,7	0,4	6,0	6,2	5,9	5,8	5,6	1,9	1,9	1,9	2,5	2,5
29.04.2021	**119**	**6**	**42**	2,5	2,6	5,9	3,7	9,7	0,0	0,2	0,1	0,2	0,1	11,1	11,3	11,0	10,7	10,6	1,9	2,5	1,9	1,9	1,9
06.05.2021	**126**	**7**	**49**	0,4	1,2	2,3	1,9	5,4	0,8	0,8	0,9	1,0	1,6	7,9	8,0	8,2	7,9	8,1	55,4	53,5	57,3	59,8	79,6
12.05.2021	**132**	**8**	**55**	0,6	0,6	3,3	1,7	6,1	0,0	0,0	0,0	0,0	0,0	14,6	14,4	14,4	14,3	14,2	55,4	54,7	54,1	57,3	61,8
20.05.2021	**140**	**9**	**63**	0,4	0,4	1,5	1,7	6,0	0,0	0,0	0,0	0,0	0,0	11,2	10,9	11,1	10,9	11,0	12,1	12,7	12,7	12,7	16,6
28.05.2021	**148**	**10**	**71**	0,9	0,0	1,8	2,4	10,1	0,0	0,0	0,0	0,0	0,0	11,7	11,5	11,8	11,5	11,7	16,6	19,1	20,4	19,1	20,4
02.06.2021	**153**	**11**	**76**	1,4	1,4	2,8		6,9	0,0	0,0	0,0	0,0	0,0	16,4	16,0	16,0	15,9	15,4	0,0	0,0	0,0	0,0	0,0
09.06.2021	**160**	**12**	**83**	1,9	2,0	4,1	3,9	10,3	0,5	0,6	0,6	0,7	0,9	17,3	17,0	17,1	16,9	16,9	54,7	54,7	54,1	54,1	71,9
16.06.2021	**167**	**13**	**90**	2,9	3,3	5,1	5,6	13,0	0,0	0,0	0,1	0,0	0,1	21,8	21,3	21,2	21,3	20,8	0,0	11,5	8,3	0,0	12,1
24.06.2021	**175**	**14**	**98**	4,4	4,8	6,7	5,9	12,2	0,2	0,2	0,2	0,2	0,2	23,8	23,0	23,1	23,0	22,6	61,1	54,7	53,5	44,6	54,7
30.06.2021	**181**	**15**	**104**	3,0	3,1	5,2	5,4	12,5	0,4	0,3	0,3	0,3	0,3	18,9	18,7	19,1	18,8	18,7	92,9	75,1	82,8	68,8	89,1
07.07.2021	**188**	**16**	**111**	1,8	2,0	2,4	3,8	10,0	0,4	0,2	0,2	0,2	0,2	17,9	17,7	17,9	17,8	17,5	57,3	54,7	47,1	53,5	44,6
15.07.2021	**196**	**17**	**119**	1,8	2,7	3,3	3,2	8,5	0,7	0,4	0,3	0,4	0,1	17,7	17,6	17,9	17,6	17,7	107	112	79,6	99,3	94,2
21.07.2021	**202**	**18**	**125**	1,6	2,2	2,2	5,6	10,0	0,0	0,1	0,1	0,1	0,0	20,0	19,7	19,8	19,8	19,3	11,5	11,5	12,1	10,8	10,2
29.07.2021	**210**	**19**	**133**	1,7	2,0	3,4	4,6	10,1	0,0	0,0	0,1	0,0	0,0	20,9	21,1	21,1	21,1	20,8	8,3	8,3	8,3	8,9	8,9
05.08.2021	**217**	**20**	**140**	1,6	1,0	2,3	3,5	10,6	0,1	0,1	0,1	0,2	0,1	17,1	17,2	17,3	17,3	17,0	31,8	30,6	29,9	31,8	30,6
11.08.2021	**223**	**21**	**146**	1,1	1,1	2,2	3,1	9,3	0,1	0,1	0,0	0,1	0,1	18,5	18,7	18,5	18,6	17,7	15,9	15,9	15,3	14,6	14,6
18.08.2021	**230**	**22**	**153**	2,6	3,4	4,6	5,8	13,2	0,1	0,3	na	0,1	0,1	20,7	21,1	20,9	21,0	20,1	14,6	19,1	na	17,8	17,2
25.08.2021	**237**	**23**	**160**	1,6	2,2	4,0	3,9	11,4	0,1	0,1	0,1	0,1	0,1	17,4	17,7	17,8	17,5	17,2	10,8	10,2	10,2	11,5	11,5
02.09.2021	**245**	**24**	**168**	1,2	2,3	3,3	3,2	7,0	0,3	0,2	0,3	0,3	0,3	13,7	13,8	14,0	13,7	13,6	57,3	57,3	73,2	63,7	63,7
09.09.2021	**252**	**25**	**175**	2,3	2,4	6,2	3,4	7,3						18,7	19,2	19,2	18,4	17,2	0,0	0,0	0,0	0,0	0,0
16.09.2021	**259**	**26**	**182**	2,1	2,0	5,8	3,0	8,5	0,1	0,0	0,0	0,0	0,0	18,6	18,8	18,9	18,3	17,7	12,7	9,5	9,5	10,2	9,5
22.09.2021	**265**	**27**	**188**	2,0	2,0	8,1	4,1	8,6	0,0	0,0	0,0	0,0	0,0	13,4	13,9	14,0	13,6	12,8	6,4	3,8	4,5	4,5	4,5
29.09.2021	**273**	**28**	**195**	1,6	1,7	7,0	2,7	7,7	0,0	0,0	0,0	0,0	0,0	15,0	15,4	15,3		13,8	5,7	5,7	5,7	6,4	6,4
06.10.2021	**279**	**29**	**202**	1,3	1,5	4,8	3,0	9,6	0,1	0,1	0,1	0,1	0,1	13,8	13,9	14,2		13,2	17,8	28,0	17,8	19,1	19,1
13.10.2021	**286**	**30**	**209**	0,9	1,1	4,2	2,6	6,6	0,1	0,1	0,0	0,1	0,0	8,2	8,4	8,4		7,6	12,7	14,6	10,2	10,8	11,5
mean/sum				**1,8**	**2,0**	**4,7**	**3,7**	**9,4**	**3,2**	**3,1**	**2,6**	**2,9**	**2,9**	**14,5**	**14,6**	**14,6**	**14,6**	**14,1**	**757**	**761**	**712**	**723**	**811**

3.3.2.2 Plant responses

Plants that had been fumigated in 2020, i.e. two-year-old plants, as well as those dedicated for the season 2021, i.e. the annual plants, were brought to ULI from March to June 2021 and were harvested from July to October 2021 one after the other considering their different life cycles. Weekly ammonia concentrations and the duration of field exposure for the 13 plant species are given in Figure 16. During the season, different measurements were taken at suitable plant species and the aboveground biomass was cut at the harvest. When feasible, we distinguished between green and senescent biomass or between different fractions such as stem, leaves and reproductive organs.

Figure 16: Measured NH_3 concentrations over the season 2021 and periods of plant exposure

NH_3 was measured with Radiello® passive samplers on a weekly basis. The dots represent measurement dates. Species are sorted according to their respective onset of exposure; above the perennials, thereunder the annuals. Colours for the five treatments follow the same colour scheme used throughout the report.



Source: Own illustration, University of Hohenheim.

Details on the different species will be given in the following sub-chapters. This season, no intermediate harvest (IMH) had been performed, but we analysed plants with/without

intermediate harvest from last year independently. When no statistically significant difference between cut and uncut plants could be observed, all ten replicates were analysed together. Unfortunately, we were not able to realize a different water supply, i.e. a "dry treatment" (DT) of one third because of the rather wet conditions during the summer of 2021.
For all parameters measured (Table 13), an analysis of variance was conducted with R studio. Ammonia and water treatments were set as factors and it was checked whether plants that had undergone an intermediate harvest in the previous season responded differently in comparison to those that had not received an intermediate cut. For this purpose, intermediate harvest was set as another factor and when the analysis showed a significant response, the plants were looked at independently. When no difference could be seen, all ten replicates were combined but "water treatment" remained a separate factor.

Table 13: Overview of all parameters and respective dates throughout the two seasons

Above: species from 2020, below: species from 2021. "HEI" is plant height or plant length, "FLON" is flower numbers. "DW" is dry weight, "GREEN" is green mass and "SEN" is senescent leaf mass. Abbreviations of parameters are given in Annex 2.

Plant Species	***Antennaria***	***Arnica* (both)**	***Carex***	***Diant. d.***	***Koeleria***	***Molinia***	***Pulsatilla***
Parameter							
Survival		04.05.2021					
HEI1	07.07.2021		06.10.2021	25.08.2021	18.08.2021	18.08.2021	08.04.2021
HEI2						29.09.2021	22.04.2021
FLON1	09.06.2021	02.06.2021			18.08.2021	29.07.2021	01.04.2021
FLON2	07.07.2021	09.06.2021					08.04.2021
FLON3							15.04.2021
FLON4							22.04.2021
FLON5							20.05.2021
SHOOTN						29.09.2021	
SEN DW		30.06.2021					22.04.2021
		02.09.2021					06.10.2021
		13.10.2021					
LEAF DW		13.10.2021			13.10.2021	29.09.2021	
		13.10.2021			13.10.2021	(green+sen)	
STEM DW						29.09.2021	
ROOT DW						29.09.2021	
TOT	07.07.2021	13.10.2021	06.10.2021	25.08.2021	13.10.2021	29.09.2021	06.10.2021
Plant Species	***Diant. g.***	***Bromus***	***Erodium***	***Iberis***	***Legousia***	***Trifolium***	
Parameter							
HEI1	20.05.2021		09.06.2021	20.05.2021	15.07.2021	22.09.2021	
HEI2	18.08.2021		30.06.2021	09.06.2021			
HEI3				21.07.2021			
FLON1	02.06.2021		09.06.2021	21.07.2021	15.07.2021	11.08.2021	
FLON2	09.06.2021		30.06.2021			22.09.2021	
FLON3	12.07.2021						
FLON4	18.08.2021						
FLODW						22.09.2021	
SHOOT DW						22.09.2021	
SEN DW		16.09.2021					
GREEN DW		16.09.2021					
TOT (aboveground) DW	18.08.2021	16.09.2021	30.06.2021	21.07.2021	15.07.2021	22.09.2021	

For each species, we are presenting the parameter total shoot mass in detail because we assessed the growth capacity of a species as its most important feature with regard to its competitive behaviour. Other parameters will be shown in tables per species and will be discussed in the subchapters. Not all data will be shown because parameters like plant length and flower number were often too variable. The exposure time and mean NH_3 concentrations during the respective time per species are shown in Table 14.

Table 14: Exposure time, produced shoot biomass and mean NH_3 concentrations for each species

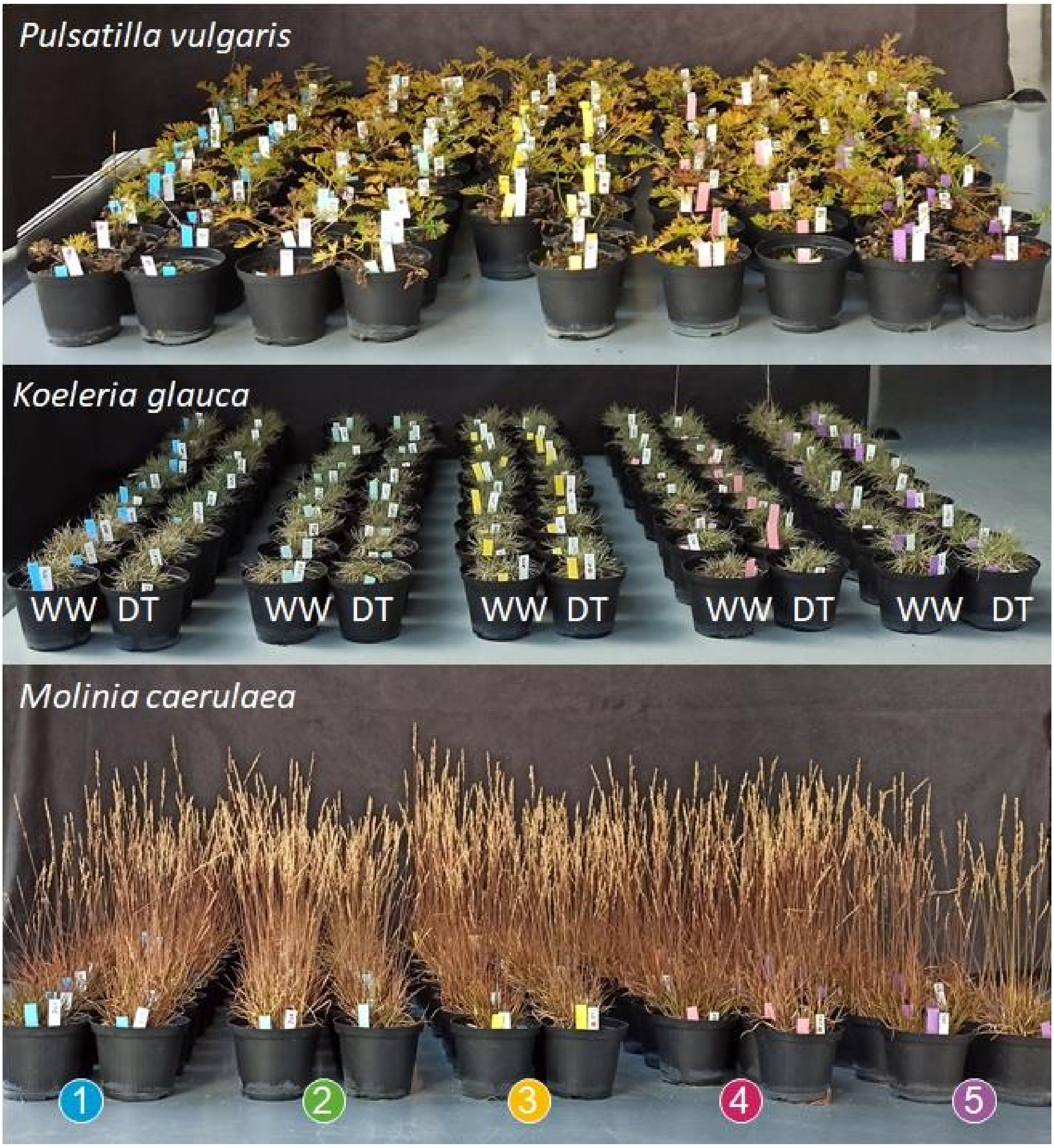
Above: species from 2020, below: species from 2021, "NH_3" represents mean ammonia concentration in in µg m^{-3} and "DM" dry matter in g. Bold numbers in the end of each block give the Mean dry weights across all treatments.

Greenhouse study 2020	***Antennaria***		***Arnica* J**		***Arnica* W**		***Carex***		***Diant. d.***		***Koeleria***		***Molinia***		***Pulsatilla***	
Start	30.04.2020		30.04.2020		30.04.2020		30.04.2020		30.04.2020		30.04.2020		30.04.2020		30.04.2020	
End	14.10.2020		14.10.2020		14.10.2020		14.10.2020		14.10.2020		14.10.2020		14.10.2020		14.10.2020	
Duration (d)	167		167		167		167		167		167		167		167	
Treatments	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM
1	5,2	0,52	5,2	3,45	5,2	1,92	5,2	1,96	5,2	2,03	5,2	3,22	5,2	1,34	5,2	3,08
2	4,3	0,57	4,3	2,69	4,3	2,18	4,3	1,58	4,3	1,79	4,3	2,51	4,3	1,12	4,3	3,09
3	5,5	0,47	5,5	2,92	5,5	2,05	5,5	1,63	5,5	1,53	5,5	2,3	5,5	1,25	5,5	3,09
4	8,4	0,58	8,4	3,07	8,4	2,35	8,4	1,63	8,4	1,6	8,4	2,67	8,4	1,29	8,4	3,33
5	17,6	0,48	17,6	3,19	17,6	2,04	17,6	1,57	17,6	1,63	17,6	2,84	17,6	1,35	17,6	3,44
		0,52		**3,06**		**2,11**		**1,67**		**1,72**		**2,71**		**1,27**		**3,21**
Field gradient study 2021	***Antennaria***		***Arnica* J**		***Arnica* W**		***Carex***		***Diant. d.***		***Koeleria***		***Molinia***		***Pulsatilla***	
Start	25.03.2021		06.05.2021		06.05.2021		24.06.2021		30.06.2021		25.03.2021		28.05.2021		18.03.2021	
End	07.07.2021		13.10.2021		13.10.2021		06.10.2021		25.08.2021		13.10.2021		29.09.2021		06.10.2021	
Duration (d)	104		160		160		104		56		202		124		202	
Treatments	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM
1	1,9	0,86	1,8	2,61	1,8	2,64	1,9	3,25	1,7	1,16	1,8	0,95	2	1,98	1,8	1,77
2	2	0,59	2	1,99	2	2,82	2,1	3,12	2,1	1,18	2	0,75	2,3	2,38	2	1,65
3	4,9	0,9	4,1	2,85	4,1	2,37	4,3	3,24	3,1	1,03	4,6	0,81	4,4	2,72	4,8	1,83
4	3,6	0,8	3,7	3,4	3,7	1,31	4	2,64	4,2	1,17	3,7	0,85	4,2	2,75	3,7	1,66
5	9,7	0,73	9,4	4,29	9,4	3,32	9,6	2,88	10,4	0,86	9,4	0,84	9,9	2,94	9,5	1,65
		0,78		**3,03**		**2,49**		**3,03**		**1,08**		**0,84**		**2,55**		**1,71**
Annuals	***Diant. gra.***		***Bromus***		***Erodium***		***Iberis***		***Legousia***		***Trifolium***					
Start	12.05.2021		06.05.2021		18.03.2021		12.05.2021		09.06.2021		24.06.2021					
End	18.08.2021		16.09.2021		30.06.2021		21.07.2021		15.07.2021		22.09.2021					
Duration (d)	98		133		104		70		36		90					
Treatments	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM	NH_3	DM				
1	1,9	6,27	1,8	0,31	1,8	0,21	2	0,43	2,8	0,07	1,9	1,39				
2	2,1	7,35	2	0,37	2	0,21	2,2	0,37	3,2	0,09	2,2	1,63				
3	3,4	7,34	3,7	0,35	5,2	0,26	3,5	0,38	4,6	0,1	4,1	1,51				
4	4,2	6,51	3,9	0,37	3,6	0,25	4,2	0,43	4,8	0,08	4,1	1,65				
5	10,2	5,8	9,6	0,47	9,7	0,3	10	0,55	11,2	0,07	9,8	0,95				
		6,65		**0,37**		**0,24**		**0,43**		**0,08**		**1,42**				

At the final harvest, all plants were sorted and placed in front of a black cloth in order to take photographs. Well-watered plants from 2020 were placed left and dry-treated plants right, starting with the control (blue labels) on the very left until the highest concentrations (purple labels) on the right side (see Figure 17).

Figure 17: Appearance of *Pulsatilla vulgaris*, *Koeleria glauca* and *Molinia caerulea* at their final harvest

From left to right: Control (blue labels) to the highest concentration (purple labels). First row per colour are plants from the previous "WW" treatment and second row are "DT" plants. From top to bottom: *Pulsatilla vulgaris*, *Koeleria glauca* and *Molinia caerulea.*

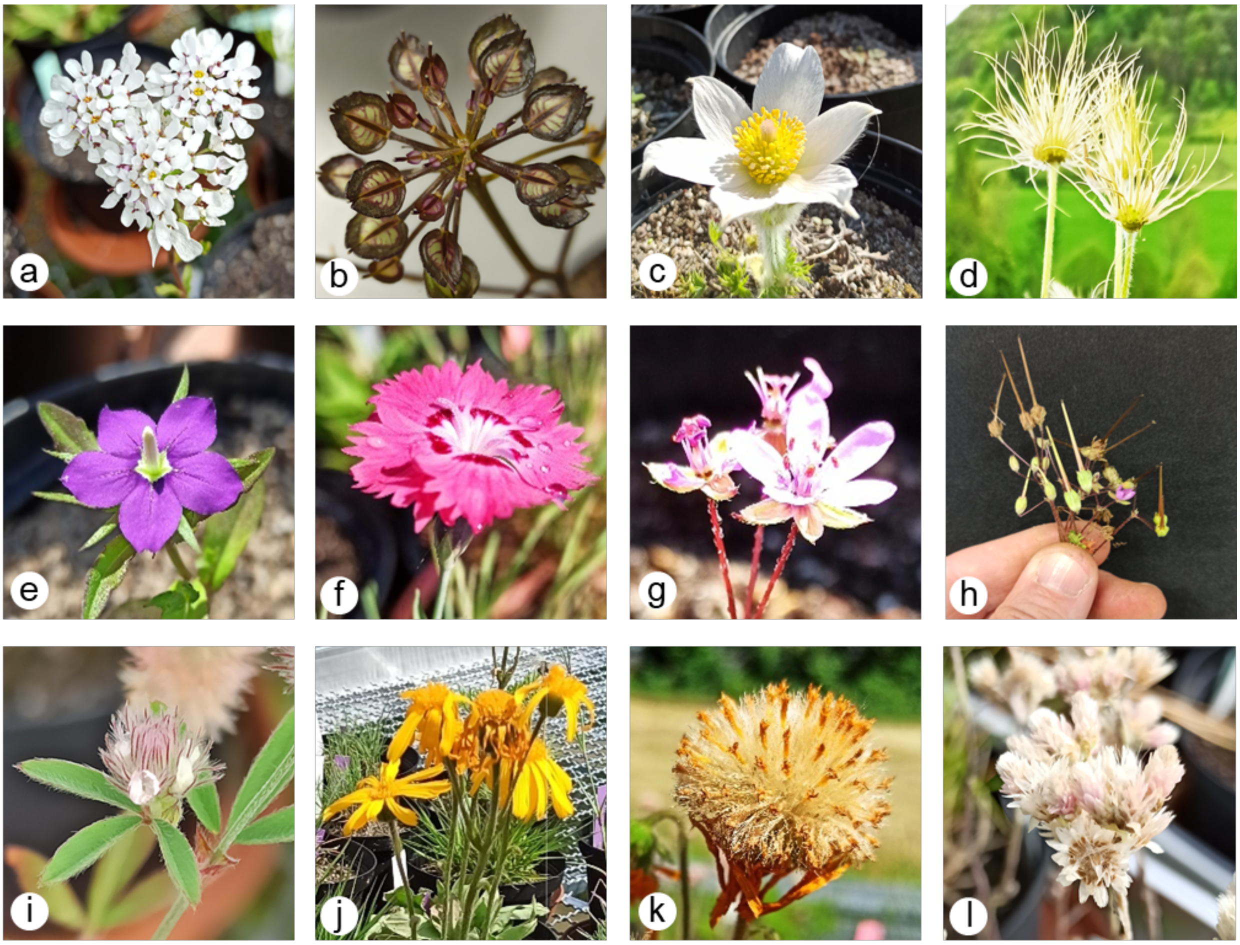


Source: Own photographs and illustrations of station identifiers. Colours for the five treatments follow the same colour scheme used throughout the report, University of Hohenheim.

Almost all species completed their life cycles and produced flowers and seeds (in contrast to most species in the greenhouse 2020). A selection of photos showing reproductive organs is presented in the following figure (Figure 18).

Figure 18: Flowering and seed ripening in some species in the season 2021

a) and b) flowering and seed ripening of *Iberis amara*; c) and d) full flowering and flower shedding in *Pulsatilla vulgaris*; e) flowering of *Legousia speculum-veneris*; f) flowering of *Dianthus gratianopolitanus*; g) and h) flowering and seed stalks of *Erodium cicutarium*; i) flowers of *Trifolium arvense*; j) and k) flowering and seed ripening of *Arnica montana*; l) flowers of *Antennaria dioica*



Source: Own photographs (the author), University of Hohenheim.

3.3.2.2.1 Plant species from the previous season (fumigation 2020)

3.3.2.2.1.1 Pulsatilla vulgaris

Pulsatilla plants were brought to ULI on 18 March 2021 and were harvested 202 days later on 6 October 2021. The mean ammonia concentrations during that period were 1.8, 2.0, 4.8, 3.7 and 9.5 µg m^{-3} for stations 1 to 5, respectively. During the season, plant height and flower numbers were measured several times and senescent material was collected once (SEN6).

At the final harvest, the aboveground biomass was cut and sorted into green and senescent material (SEN7), dried and weighed. The flower number per plant did not differ between ammonia or water treatments until the 4th measurement (FLON4) on 22 April 2021, when a significant water effect could be observed (Table 15).

Since no water deficit could be realized during the season 2021, we refer to water effects in 2021 as carry-over effects from 2020. The two highest ammonia treatments showed the biggest difference of "moist" and "dry" flower numbers and some weeks later, on 20 May 2021 when flower number was determined the fifth time, the effect was even more pronounced: plants from nearly all ammonia treatments showed a lower number of flowers in the plants that were subjected to dry conditions in the previous season. With regard to the plant length, no effects could be seen at the first determination (8 April 2021), but only two weeks later, a significant water effect could be observed. DT plants were generally shorter than those which were subjected to a higher water supply in the previous season. No differences between the ammonia stations could be seen in flower numbers and length, but plants had only been exposed for about two months until then.

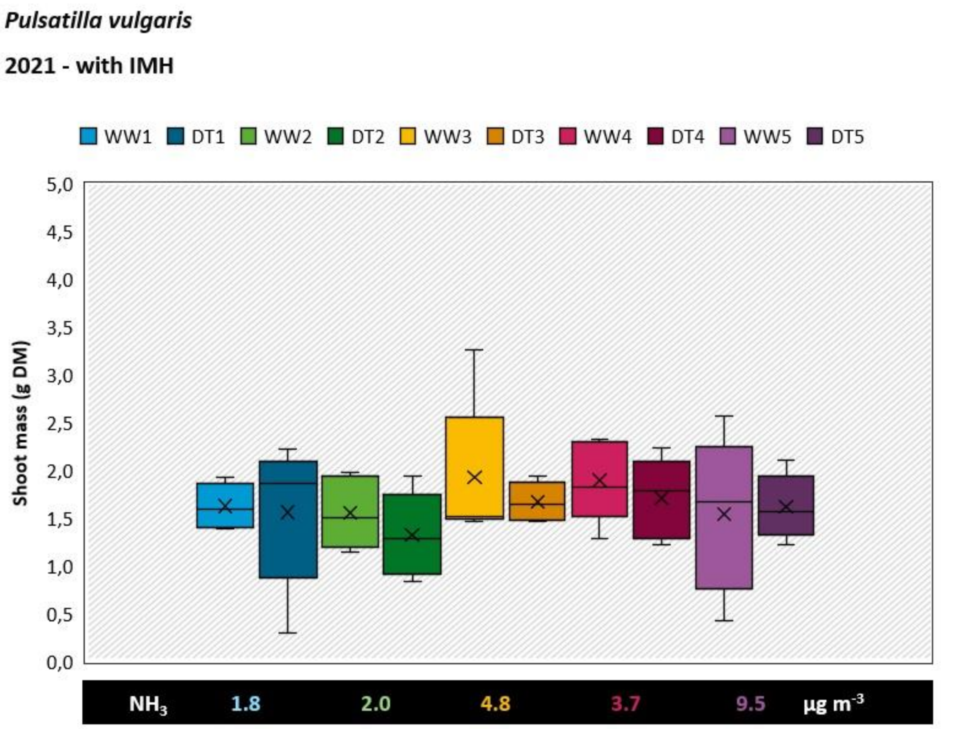
On the 22 April 2021, senescent plant material was collected from the plants (SEN6). Because of an effect of the intermediate harvest (IMH), plants were looked at independently. Plants that had been cut twice before (SET1) showed a higher production of senescent plant material and a significant ammonia effect in treatment 5 that was exposed closest to the farm: treatment 5 produced a lower amount of senescent biomass than treatment 1.

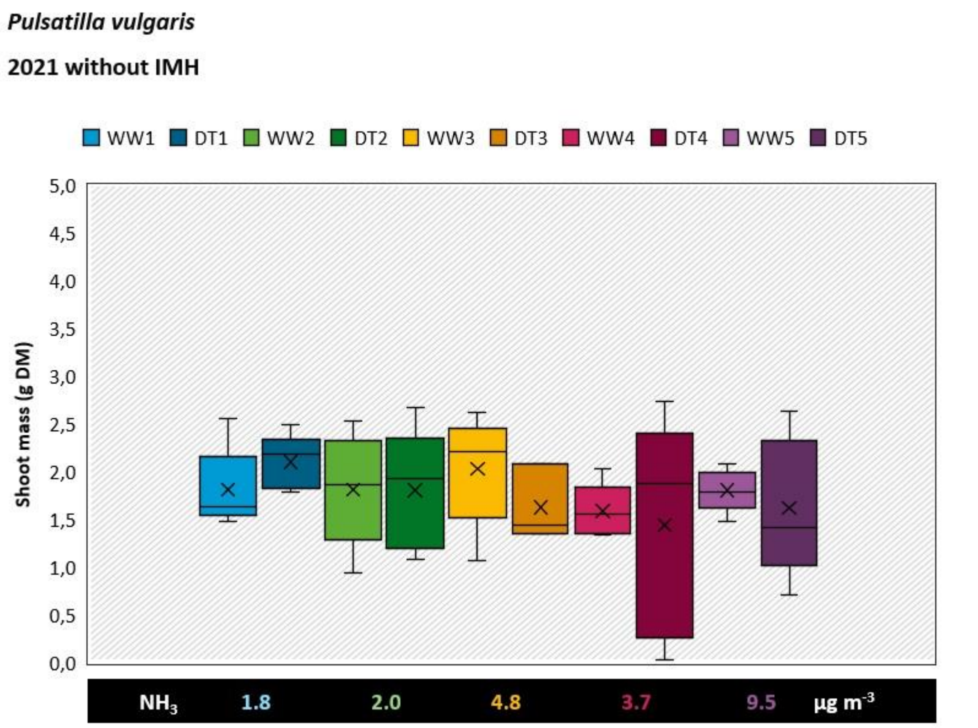
For plants without a previous intermediate harvest, no treatment but an interaction effect could be seen. At the final harvest, the senescent fraction (SEN7) did not show differences between the treatments (Table 15), but plants without intermediate harvest produced generally more biomass than the other plant group. The green fraction (data not shown) did not differ between SETs, but the ANOVA revealed an interaction effect of water and ammonia. The total shoot mass (all senescent plus the green fraction) showed no differences between the treatments, but again plants without an IMH had been producing more biomass (Figure 19) suggesting that cutting (mowing) the plants in the previous season removed resources that would have been needed in the following year. In total, the biomass produced in 2021 was lower than in previous season. This is due to the fact that pots were not fertilized in the season 2021 and plants were kept in very nutrient-poor, now impoverished soil.

For *Pulsatilla vulgaris*, we can therefore state that the different low ammonia concentrations seemed to not have affected the plants. Neither in 2020 when plants were kept in the greenhouse, nor in the natural field gradient, plants reacted more than sporadically to the different ammonia concentrations. The drought induced in 2020 had no effects on the plants in the same year, but slight effects such as less flowers and a reduced height could be observed in the following season. Generally, we assess the species as rather drought resistant because effects were not as pronounced as in the other species and the total shoot production was not much affected.

Figure 19: Total shoot mass of *Pulsatilla vulgaris* with/without previous intermediate harvest

Shoot mass was determined on 6 October 2021. Colour codes indicate the arrangement of station numbers after ammonia levels. Lighter colours = WW (well-watered in 2020); Darker colours=DT (dry treatment in 2020). Left: plants with IMH in 2020, right: plants without IMH in 2020. Numbers on x-axis indicate the respective mean ammonia concentrations (µg m-3).





Source: Own illustrations, University of Hohenheim.

Table 15: *Pulsatilla vulgaris* parameters measured throughout the season 2021

"FLON"= Flower Number; "HEI"=Length [cm]; "SEN"=Senescent Biomass [g]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Add-ons -indicate whether 1. the plants were subjected to an intermediate harvest (IMH) or 2 were not harvested during the season without IMH). Numbers 1 to 5 indicate the ammonia treatments; "WW"= Well watered in 2020; "DT"= Dry treatment from 2020 (-33% watering). Different letters within one column indicate statistical differences as identified by Tukey´s HSD tests. In the rows underneath, it is noted which of the treatments were different from each others, e.g. "FLON5" was significantly higher in treatment 1 as compared to treatment 2.

NH_3	Water	FLON5	HEI1	HEI2	SEN6-1	SEN6-2	SEN7-1	SEN7-2	SHOOT-1	SHOOT-2
1	WW	2.9±2.1 a	5.6±2.0 a	9.6±2.8 a	0.3±0.1 ab	0.1±0.1 ab	0.6±0.1 a	1.1±0.3 a	1.6±0.2 a	1.8±0.4 a
	DW	1.6±1.0 ab	4.2±2.1 a	7.8±3.2 a	0.5±0.1 a	0.2±0.1 a	0.6±0.2 a	1.0±0.3 a	1.6±0.7 a	2.1±0.3 a
2	WW	1.4±1.1 ab	5.3±1.9 a	8.7±4.2 a	0.3±0.1 ab	0.2±0.1 ab	0.4±0.2 a	1.0±0.4 a	1.7±0.3 a	1.8±0.5 a
	DW	0.8±0.7 b	5.5±2.1 a	8.8±3.3 a	0.3±0.1 ab	0.1±0.1 ab	0.6±0.2 a	0.8±0.4 a	1.3±0.4 a	1.8±0.6 a
3	WW	2.1±1.8 ab	6.0±2.3 a	9.2±4.2 a	0.2±0.1 bc	0.1±0.1 b	0.9±0.4 a	0.8±0.3 a	1.9±0.7 a	2.0±0.5 a
	DW	2.1±0.9 ab	6.1±2.0 a	10.3±3.2 a	0.3±0.1 ab	0.4±0.2 ab	0.8±0.4 a	0.5±0.3 a	1.7±0.2 a	1.6±0.3 a
4	WW	2.5±1.4 ab	5.2±1.4 a	9.5±2.8 a	0.4±0.2 ab	0.1±0.0 ab	0.8±0.2 a	0.8±0.1 a	1.9±0.4 a	1.6±0.2 a
	DW	1.0±1.1 ab	3.8±1.3 a	5.8±2.1 a	0.3±0.1 ab	0.3±0.2 ab	0.9±0.4 a	1.0±0.4 a	1.7±0.4 a	1.4±1.0 a
5	WW	2.5±1.0 ab	5.1±1.6 a	8.6±2.5 a	0.2±0.1 c	0.1±0.1 b	0.6±0.5 a	0.8±0.1 a	1.5±0.7 a	1.8±0.2 a
	DW	1.1±1.4 ab	4.6±2.4 a	6.4±2.9 a	0.2±0.0 c	0.1±0.0 b	0.7±0.3 a	0.9±0.5 a	1.6±0.3 a	1.6±0.7 a
H_2O		*1>2		*1>2						
NH_3					* 1>5					
H_2OxNH_3						*				

3.3.2.2.1.2 Koeleria glauca

Koeleria plants were brought to ULI one week after *Pulsatilla*, on 25 March 2021 and were harvested on 13 October 2021 (Figure 17), resulting in an exposure time of 202 days as well. The mean ammonia concentrations during that time for the five stations were 1.8, 2.0, 4.6, 3.7 and 9.4 µg m^{-3}.

During the season, plant length and flower number were determined only once, since it was visible already in the beginning that this species did not develop well because neither much biomass nor flowers were produced. We suggest that severe nutrient limitations were responsible for this. At the final harvest, senescent and green plant biomass were separated, dried and weighed. The flower number was determined as well, but had not changed since the first measurement in August. Only three plants (from the two highest NH_3 concentrations) produced reproductive organs, but this low number proved to be statistically insignificant (Table 16).

With regard to the plant length, a difference between cut and uncut plants could be seen. Plants that were cut twice last season, i.e. subjected to an IMH, were generally smaller but showed a significant difference between treatment 5 compared to treatments 4, 3 and 2. The other half of the plants were taller and included the three flowering plants. Furthermore, an interaction effect was detected.

With regard to the different fractions harvested, we can see the following. Senescent, green and the total shoot mass showed differences between the SETs. All fractions were heavier when no intermediate harvest took place before, indicating that we created nutrient limited conditions that would probably stimulate the plant's capacity to take up ammonia.

The production of senescent biomass in plants with an intermediate harvest did not show differences between the treatments. The other plants, however, showed a significant water and ammonia effect. Plants subjected to a DT in the previous season produced significantly less senescent biomass than the WW plants, suggesting that the plants do not shed leaf mass at very nutrient limited conditions. The ammonia effect could be detected between treatments 1 and 2 and 5: Treatment 1 (the control) produced more senescent biomass than treatments 2 and 5.

Table 16: *Koeleria glauca* parameters measured throughout the season 2021

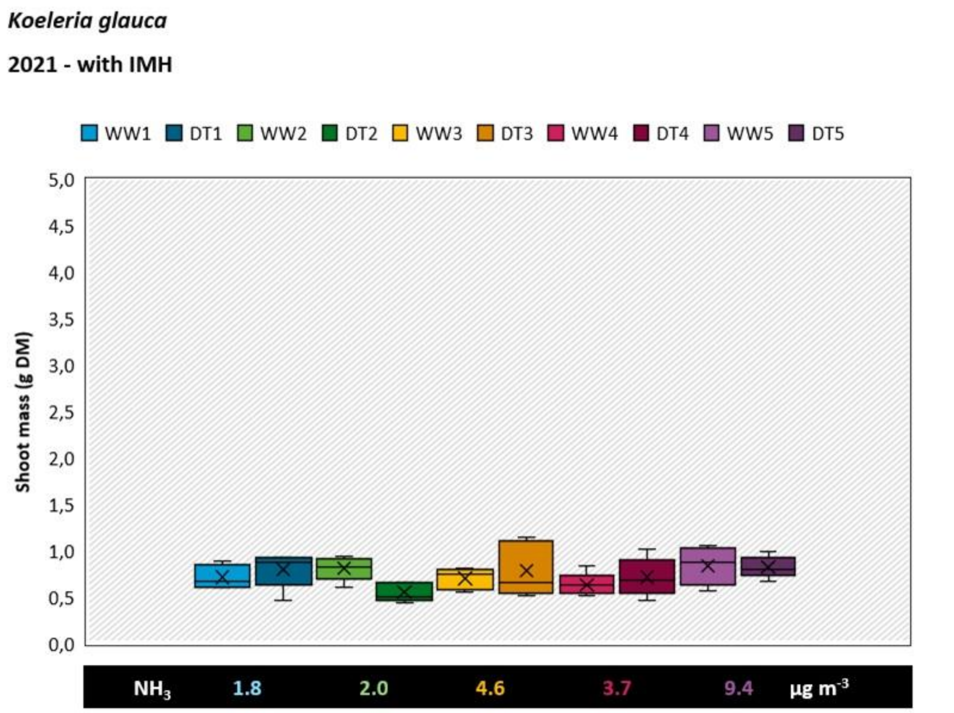
"HEI"=Length [cm]; "SEN"=Senescent Biomass [g]; "GREEN"=green leaf mass. "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Add-ons -indicate whether 1. the plants were subjected to an intermediate harvest (IMH) or 2 were not harvested during the season (without IMH). Numbers 1 to 5 indicate the ammonia treatments; "WW"= Well watered in 2020; "DT"= Dry treatment from 2020 (-33% watering). Different letters within one column indicate statistical differences as identified by Tukey´s HSD tests. In the rows underneath, it is noted which of the treatments were different from each others, e.g. "HEI" was significantly higher in treatment 5 as compared to treatments 2, 3 and 4.

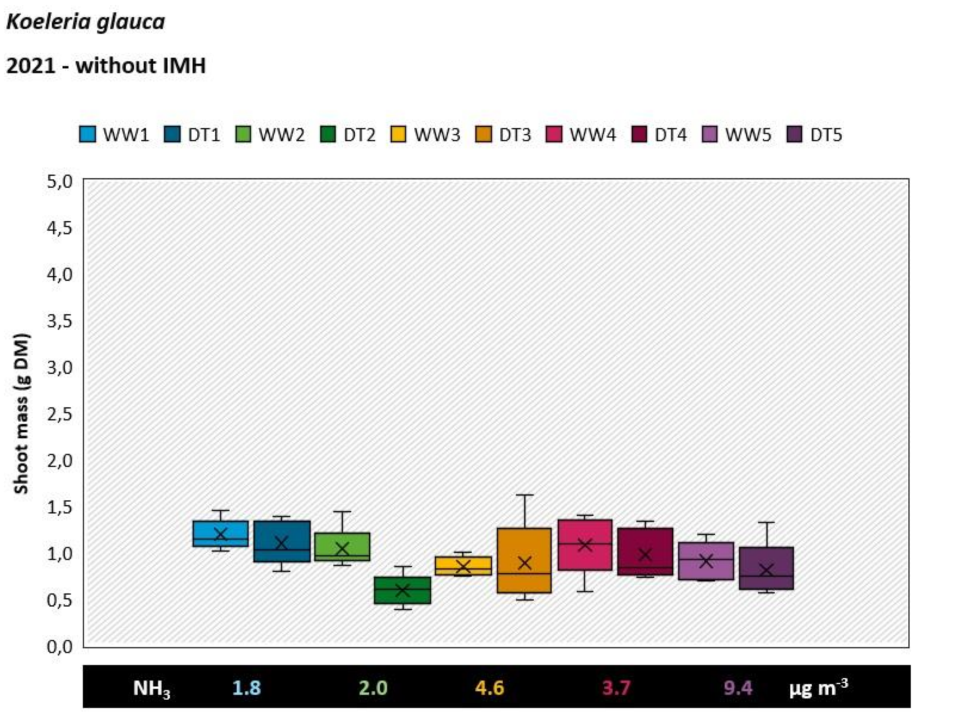
NH_3	Water	HEI-1	HEI1-2	SEN-1	SEN-2	GREEN-1	GREEN-2	SHOOT-1	SHOOT-2
1	WW	7.3±0.5 ab	8.5±0.4 ab	0.7±0.1 a	0.8±0.1 a	0.3±0.1 ab	0.4±0.1 a	0.7±0.1 a	1.2±0.1 a
	DW	7.4±1.0 ab	8.6±1.0 ab	0.4±0.1 a	0.7±0.1 ab	0.4±0.1 a	0.4±0.1 a	0.8±0.2 a	1.1±0.2 ab
2	WW	7.5±0.4 ab	7.6±0.5 a	0.5±0.1 a	0.6±0.1 ab	0.3±0.1 ab	0.4±0.1 a	0.8±0.1 a	1.0±0.2 ab
	DW	6.9±0.7 ab	6.5±0.6 a	0.3±0.1 a	0.3±0.1 b	0.3±0.0 b	0.3±0.1 a	0.6±0.1 a	0.6±0.2 b
3	WW	6.8±0.7 ab	7.1±0.9 a	0.4±0.0 a	0.5±0.1 ab	0.3±0.1 ab	0.4±0.0 a	0.7±0.1 a	0.8±0.1 ab
	DW	7.5±1.1 ab	8.0±1.0 ab	0.5±0.2 a	0.5±0.3 ab	0.3±0.1 ab	0.4±0.1 a	0.8±0.3 a	0.9±0.4 ab
4	WW	7.1±0.7 ab	7.8±0.5 a	0.4±0.1 a	0.7±0.2 ab	0.3±0.1 ab	0.4±0.1 a	0.6±0.1 a	1.1±0.3 ab
	DW	6.3±0.7 a	11.8±9.1 ab	0.4±0.1 a	0.6±0.2 ab	0.3±0.1 ab	0.4±0.1 a	0.7±0.2 a	1.0±0.2 ab
5	WW	8.5±1.4 b	17.7±9.3 b	0.4±0.1 a	0.5±0.1 ab	0.4±0.1 ab	0.4±0.1 a	0.8±0.2 a	0.9±0.2 ab
	DW	8.5±0.4 b	8.1±0.2 ab	0.5±0.1 a	0.4±0.2 ab	0.±0.1 ab	0.4±0.1 a	0.8±0.1 a	0.8±0.3 ab
H_2O					*1>2				
NH_3		*5>2,3,4			*1>2,5				* 1>2
H_2OxNH_3		*							

The green shoot mass of plants with an intermediate harvest in 2020 did not show a significant ammonia effect, but the post-hoc test revealed differences between single treatments (treatments 1 and 2). Still it can be seen that the highest ammonia concentration yielded the highest green plant biomass. Plants without an intermediate harvest did not show any differences between the treatments. The sum of green and senescent biomass amounts to the total shoot mass. The plants with previous intermediate harvest did not show any differences with regard to the shoot mass and produced less biomass than the other plants. The uncut plants showed an ammonia effect between treatment 1 and 2 (1>2), but not between any other treatments (Figure 20).

Figure 20: Total shoot mass of *Koeleria glauca* with/without previous intermediate harvest

Shoot mass was determined on 13 October 2021. Colour codes indicate the arrangement of station numbers after ammonia levels. Lighter colours = WW (well-watered in 2020); Darker colours=DT (dry treatment in 2020). Left: plants with intermediate harvest (IMH) in 2020, right: plants without IMH in 2020. Numbers on the x-axis indicate the respective mean ammonia concentrations (µg m^{-3}).





Source: Own illustrations, University of Hohenheim.

3.3.2.2.1.3 Molinia caerulea

Molinia plants were exposed at ULI after 28 May 2021 for 124 days until the harvest on 29 September 2021. The mean ammonia concentrations during that time were 2.0, 2.3, 4.4, 4.2 and 9.9 µg m^{-3}. Throughout the season, plant length and the flower number were measured once. At the final harvest, plant length was determined again, the number of stalks was counted and the aboveground biomass was separated into stems and leaves. Additionally, the roots were washed, dried and weighed (Figure 21). Root mass was looked at because we wanted to compare the total plant weight of the ramets from the beginning and the end of the experiment. In the very beginning of the experiment in early 2020, *Molinia* clones were uniformly cut into pieces of 10g fresh matter at the start. Total dry mass in the end was 18 g on average, meaning that over the experiment biomass of the ramets increased at least ninefold.

Figure 21: Washing of *Molinia caerulea* roots after the final harvest on 29 September 2021

Sand was carefully shaken out from roots which were then washed thoroughly with a sprinkler

Source: Own photographs from the author, University of Hohenheim.

For the first parameter, flower number, we had to distinguish between plants that had undergone an intermediate harvest last year (add on 1) and the ones that had not been cut during the season (add on 2). Plants from first group did not show an ammonia effect but an interaction effect of the NH_3 and water treatments could be detected. In contrast, previously uncut plants showed a significant NH_3 effect: treatment 5 produced significantly more flowers than treatment 1 (Table 17). However, we do not suspect this as a big competitive advantage due to the fact that the *Molinia* propagation mainly happens vegetatively via ramets.

With regard to the plant height, previously cut and uncut plants differed as well. Plants without previous intermediate harvest were generally taller than the others. For both groups, an interaction effect of water and ammonia treatment could be seen. The fractions stem, leaf, root and total shoot were generally larger when no intermediate harvest took place in the preceding

season. The aboveground fractions stem, leaf and total shoot each showed a significant interaction effect for plants with an intermediate harvest: DT seemed to boost the ammonia effect (not significantly), whereas in the WW almost no differences were visible (Table 17, Figure 22). For the total shoot mass, the plants without the intermediate harvest showed a significant ammonia effect in all fractions. The shoot mass of treatments 3, 4 and 5 was significantly higher than that from treatment 1. Furthermore, shoot mass from treatment 5 was higher than that from treatment 2.
Interestingly, plants with IMH only revealed an ammonia effect regarding the shoot production in the DT treatment (interaction effect). It might be that the mowing and additional watering in combination decreased the nutrient status in the soil drastically with the result that re-growth in the following season was very limited and not even the highest ammonia concentrations could compensate the deficiency. Further comments on the nutrient economy of that species are made in chapter 3.4.
The fraction root did not reveal any effects for plants from SET1, but a water effect for SET2 could be seen: plants from the DT showed a lower root mass than the WW treatment. This pattern can be transferred to the total biomass, i.e. the sum of all fractions (Table 17).

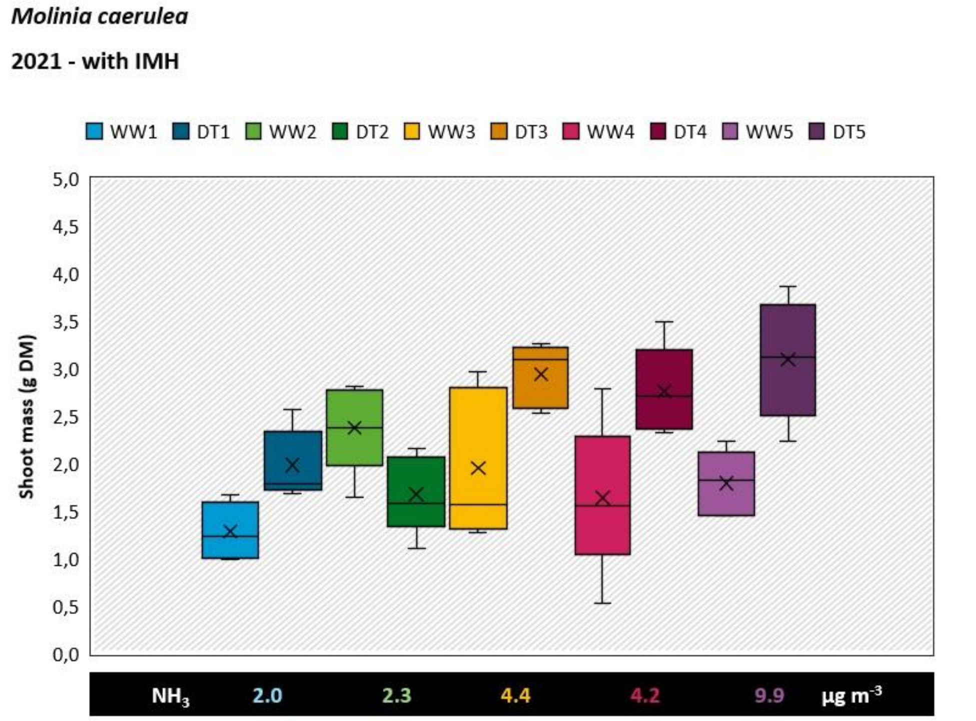
Table 17: *Molinia caerulea* parameters measured throughout the season 2021

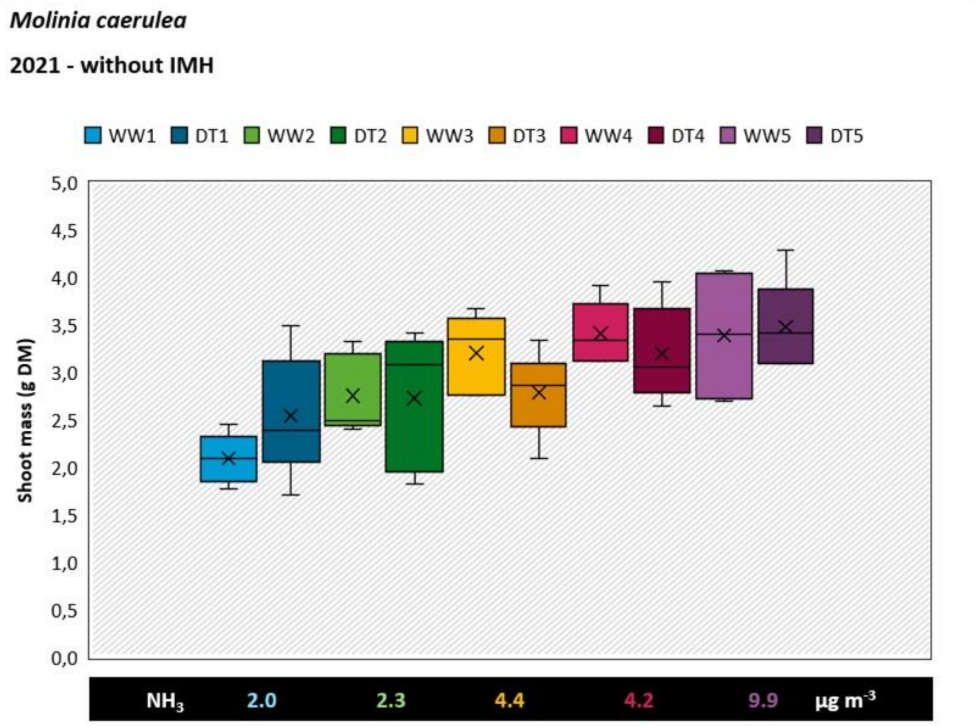
"FLON"=Flower Number; "HEI"=Length [cm]; "STEM"=Stem Dry Weight [g]; "LEAF"= Total Leaf Dry Weight [g]; "SHOOT"=Total Shoot Biomass [g]) ± standard deviation. Add-ons -1 and -2 indicate the belonging of the cut plants (1-with IMH) and uncut plants (2-without IMH). Numbers 1 to 5 indicate the ammonia treatments; "WW"= Well watered in 2020; "DT"= Dry treatment from 2020 (-33% watering). Different letters within one column indicate statistical differences between treatments using Tukey tests. In the rows underneath, the differences between individual treatments are given, e.g. FLON was significantly higher in ammonia treatment 5 as compared to treatment 1.

NH_3		FLON1-2	HEI1-1	HEI1-2	STEM-1	STEM-2	LEAF-1	LEAF-2	SHOOT-1	SHOOT-2
1	WW	14.0±3.8 a	36.5±9.2 a	41.8±3.4 ab	0.6±0.2 a	1.2±0.2 a	0.7±0.2 a	0.9±0.1 a	1.3±0.3 a	2.1±0.2 a
	DW	15.8±3.2 ab	38.4±3.2 ab	42.3±8.0 ab	1.1±0.2 ab	1.5±0.5 ab	0..9±0.1 ab	1.0±0.2 ab	2.0±0.3 a	2.5±0.6 ab
2	WW	18.6±1.9 a	44.1±4.7 ab	44.6±3.9 ab	1.3±0.1 ab	1.8±0.2 ab	1.10±0.3 ab	0.9±0.1 ab	2.4±0.4 ab	2.8±0.4 ab
	DW	18.8±5.3 a	35.5±1.8 a	39.8±1.6 a	0.9±0.2 ac	1.7±0.5 ab	0.8±0.2 a	1.1±0.2 ab	1.7±0.4 ac	2.7±0.6 ab
3	WW	17.6±3.4 a	37.1±5.7 ab	51.7±4.2 b	1.1±0.5 ab	2.1±0.3 b	0.8±0.2 ac	1.1±0.1 ab	2.0±0.7 a	3.2±0.4 b
	DW	18.6±2.0 a	44.4±5.5 ab	44.2±5.8 ab	1.8±0.2 b	1.7±0.4 ab	1.1±0.3 ab	1.1±0.1 ab	2.9±0.3 b	2.8±0.4 ab
4	WW	20.8±3.2 a	34.6±3.2 a	43.0±2.4 ab	0.7±0.5 a	2.1±0.3 b	0.9±0.3 ab	1.3±0.1 b	1.6±0.7 a	3.4±0.3 b
	DW	17.8±2.9 a	42.4±3.5 ab	50.5±2.2 b	1.6±0.4 bv	2.0±0.3 ab	1.2±0.1 c	1.2±0.1 ab	2.8±0.4 bc	3.2±0.4 b
5	WW	21.8±3.5 a	44.4±3.8 ab	46.0±2.3 ab	1.1±0.2 ab	2.3±0.4 b	0.7±0.1 a	1.0±0.2 ab	1.8±0.3 a	3.4±0.6b
	DW	18.4±3.2 a	48.7±2.8 b	49.0±5.9 ab	1.8±0.3 b	2.30±0.5 b	1.3±0.2 b	1.2±0.2 ab	3.1±0.6 b	3.5±0.4 b
H_2O										
NH_3		* 5>1				*4,5>1;5>2		*4>1,2		* 3,4,5>1;5>2
H_2OxNH_3			*	*	*		*		*	

Figure 22: Total shoot mass of *Molinia caerulea* with/without previous intermediate harvest

Shoot mass was determined on 29 September 2021. Colour codes indicate the arrangement of station numbers after ammonia levels. Lighter colours = WW (well-watered in 2020); Darker colours=DT (dry treatment in 2020). Left: plants with IMH in 2020, right: plants without IMH in 2020. Numbers on the x-axis indicate the respective mean ammonia concentrations (µg m^{-3}).





Source: Own illustrations, University of Hohenheim.

3.3.2.2.1.4 Arnica montana (both origins)

Both *Arnica* origins suffered during the extreme season 2020 in the hot greenhouse and several plants died the following winter and also during the season 2021 (Figure 23). This is why we had to deal with fewer replicates and therefore statistical analyses (ANOVA and multiple comparisons) were somewhat limited. The data shown (means per treatment and standard deviation) should thus not to be over-interpreted (Table 18). The mean NH_3 concentrations remaining plants were exposed to were 1.8, 2.0, 4.1, 3.7 and 9.4 µg m^{-3}. The flower number was determined twice (on 2 and 9 June 2021) and senescent material was collected on 30 June and 2 September (SEN6 and 7). Additionally, at the final harvest on 13 October 2021, we distinguished between green and senescent biomass (SEN8). For the parameter flower number in *Arnica* “Jelitto” plants, no clear trend can be seen. In contrast to the previous summer, *Arnica* plants from both origins developed flowers. 69,6% of the “Jelitto” plants and 75 % of the “Weiherberg” plants produced flowers. For all senescent fractions, there seems to be a carry-over effect of the drought from the preceding season: Dry plants (DT) produced less senescent (and also total shoot) biomass than plants from the WW treatment. For the sum of the senescent fractions, this carry-over effect seemed to be even more pronounced. Plants at station 5 produced more senescent and furthermore more total shoot biomass than the other treatments, but as stated above, the number of remaining replicates was too low for appropriate significance tests. *Arnica* “Weiherberg” plants did not show a clear reaction pattern to the ammonia or water treatments, but produced less shoot mass than the commercial plant origin. SEN6 was generally higher in dry plants, but SEN7 showed the opposite effect. SEN8 was lower again and this could also be seen in the sum of all senescent fractions. At station 5, nearly no more differences could be observed.

Figure 23: Appearance of the remaining *Arnica plants* at their final harvest.

From left to right: Control to the highest concentration, indicated by the colour labels. First row per colour are plants from the previous “WW” water treatment and second row are “DT” plants. Note that the remaining number of plants decreased from 100 to 45 in the “Jelitto” and 30 in the “Weiherberg” population.

Source: Own photographs from the author, University of Hohenheim.

Table 18: *Arnica montana* (two origins) parameters measured throughout the season 2021

"FLON"= Flower Number; "SEN"=Senescent Biomass [g] (6, 7 and 8=collection on 30.6, 2.9. and 13.10); "SENSUM"=Sum of all senescent fractions [g]; "GREEN"=Green Biomass [g]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments; "WW"= Well watered in 2020; "DT"= Dry treatment from 2020 (-33% watering). Note that test for statistically significant differences could not be performed due to a strongly reduced number of replicates. Note: no plants of treatment 1 had survived under DT conditions.

Arnica J		Parameter							
NH_3	Water	FLON1	FLON2	SEN6	SEN7	SEN8	SENSUM	GREEN	SHOOT
1	WW	0.3±0.5	4.9±3.1	0.6±0.6	1.4±0.5	1.6±0.5	3.6±0.8	0.3±0.2	3.9±0.7
	DT	1.0±0.0	6.0±0.0	0.0±0.0	0.3±0.0	0.4±0.0	0.5±0.0	0.7±0.0	1.3±0.0
2	WW	0.2±0.4	2.3±3.1	0.6±0.5	1.4±1.0	0.9±0.6	2.9±1.7	0.5±0.3	3.4±1.9
	DT	0.0±0.0	2.7±2.1	0.0±0.0	0.0±0.0	0.3±0.4	0.3±0.4	0.3±0.1	0.6±0.5
3	WW	0.1±0.3	2.8±2.3	0.2±0.5	1.1±0.7	2.3±0.5	3.4±1.4	0.3±0.6	4.2±1.0
	DT	0.5±0.0	4.0±0.0	1.0±0.0	0.0±0.0	1.0±0.2	1.6±0.3	0.0±0.0	1.6±0.3
4	WW	0.2±0.4	2.8±2.7	0.5±0.6	0.9±0.5	1.2±0.3	2.5±1.2	0.3±0.2	2.9±1.4
	DT	0.0±0.0	0.0±0.0	2.9±0.0	0.0±0.0	0.6±0.0	3.5±0.0	0.5±0.0	4.0±0.0
5	WW	0.2±0.4	5.8±3.4	0.7±0.7	2.1±1.1	1.6±0.5	4.4±0.9	0.7±0.1	5.1±0.9
	DT	0.0±0.0	3.6±2.3	1.0±0.6	0.9±0.5	1.5±0.6	3.2±1.2	0.3±0.2	3.4±1.2
Arnica W									
1	WW	0.3±0.5	4.7±0.9	1.1±1.1	0.4±0.3	0.9±0.1	2.4±0.8	0.2±0.2	2.6±0.7
	DT								
2	WW	0.0±0.0	5.0±1.4	0.6±0.4	1.2±0.8	1.2±0.7	2.9±1.6	0.4±0.4	3.4±1.8
	DT	0.2±0.4	2.8±2.4	1.6±1.1	0.3±0.4	0.2±0.1	2.0±0.9	0.3±0.1	2.3±1.0
3	WW	0.0±0.0	2.2±07	0.1±0.2	1.8±0.9	1.1±0.1	3.1±0.8	0.1±0.1	3.2±0.9
	DT	0.5±0.5	4.0±1.0	1.0±0.4	0.0±0.0	1.0±0.0	1.6±0.1	0.0±0.0	1.6±0.1
4	WW	0.1±0.3	2.1±2.3	0.1±0.1	0.8±0.3	0.8±0.3	1.7±0.5	0.2±.2	2.0±0.7
	DT	0.0±0.0	2.0±2.0	0.3±0.4	0.0±0.0	0.1±0.0	0.4±0.4	0.1±0.1	0.7±0.2
5	WW	0.3±0.5	4.0±2.9	0.5±0.4	0.4±0.4	1.0±0.4	2.0±0.3	0.2±0.1	2.1±0.3
	DT	0.0±0.0	1.5±1.5	1.2±1.2	0.7±0.7	0.5±0.0	2.1±2.1	0.3±0.0	4.5±0.0

The total shoot mass was generally lower in the dry treatments, but at station 5 it was the other way round. Leaf material of the species has been analysed for its ^{15}N signatures in order to further discuss the fate of ammonia in the plants and to accomplish the data set from the previous season. Data are presented in chapter 3.4.

3.3.2.2.1.5 Antennaria dioica

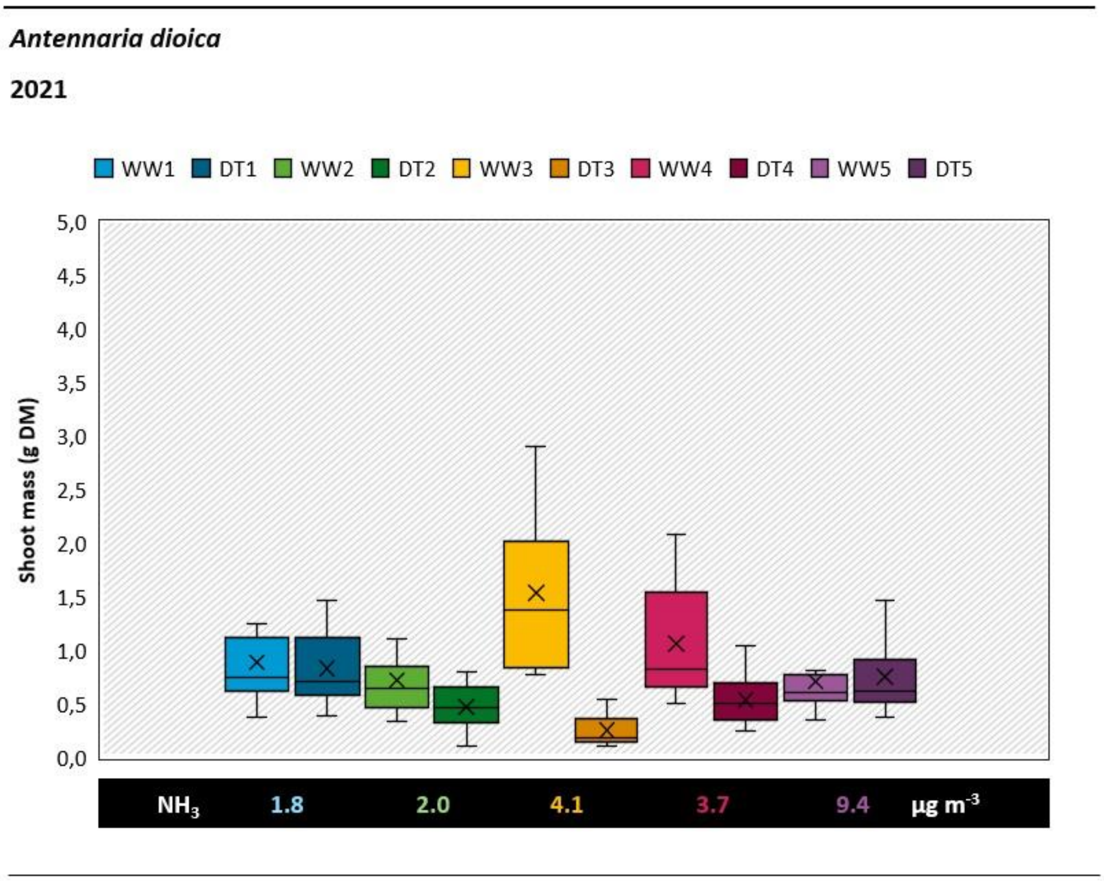
Antennaria plants were exposed on 25 March 2021 and were harvested 104 days later on 7 July 2021. The mean concentrations during that time were 1.9, 2.0, 4.9, 3.6 and 9.7 µg m^{-3} for stations 1 to 5, respectively. During the season, we twice determined the flower number and at the final harvest, we observed plant length (HEI), flower numbers (FLON) and the total aboveground biomass (SHOOT). Plants were harvested only once in 2020. Therefore, no differentiation after cutting frequency needed to be respected.

Table 19: *Antennaria dioica* parameters measured throughout the season 2021

"FLON"= Flower Number; "HEI"=Length [cm]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments; "WW"= Well watered in 2020; "DT"= Dry treatment from 2020 (-33% watering). Different letters within one column indicate statistical differences between treatments using multiple comparison Tukey tests.

NH_3	Water	Parameter FLON1	FLON2	LEN1	SHOOT
1	WW	5.6±5.0 ab	5.6±5.0 ab	15.4±4.5 a	0.9±0.4 a
	DT	3.8±2.8 ac	3.8±2.8 ac	14.9±4.7 a	0.8±0.3 ac
2	WW	3.1±3.4 ac	3.6±3.8 a	14.9±4.6 a	0.7±0.4 ac
	DT	0.3±0.7 a	0.3±0.7 a	4.5±5.0 b	0.5±0.2 ac
3	WW	10.9±6.2 b	11.1±6.4 b	18.7±1.5 a	1.5±0.7 b
	DT	0.2±0.4 a	0.2±0.4 a	4.4±4.9 b	0.3±0.1 c
4	WW	8.0±5.9 bc	8.0±5.5 b	16.9±1.6 a	1.1±0.5 ab
	DT	1.3±2.4 a	1.3±2.4 a	5.3±5.2 b	0.5±0.2 ac
5	WW	2.3±2.6 ac	2.2±2.6 a	13.1±5.8 ac	0.7±0.3 ac
	DT	1.6±1.9 a	1.6±1.9 a	10.3±6.5 bc	0.8±0.3 ac
H_2O					
NH_3					
H_2OxNH_3		*	*	*	*

Figure 24: Total shoot mass of *Antennaria dioica*



Source: Own illustration, University of Hohenheim.

Flower numbers did not increase between the two measurement dates (09 June and 07 July) and no clear pattern with regard to the ammonia and water treatments could be seen. Plant length as well as shoot biomass (Figure 24) did not show any ammonia effects, but in all parameters, interaction effects were noticed (Table 19). The results suggest that the heathland species *A. dioica* does not respond to low concentrations of ammonia. Only at higher deposition and at low pH levels the species may be prone to NH_4^+ toxicity (van den Berg et al. 2005).

3.3.2.2.1.6 Carex arenaria

Carex plants were brought to ULI rather late because plant growth only started in the early summer. They were exposed from 24 June 2021 until 6 October 2021, for 104 days in total. The mean ammonia concentrations during that period were 1.9, 2.1, 4.3, 4.0 and 9.6 µg m^{-3}. Similar to *Koeleria*, *Carex* plants did not grow much and no flower at all was produced. That's why only at the final harvest measurements were taken: We determined plant length (HEI) and the total aboveground biomass (SHOOT). With regard to the length, no differences between plants with and without previous intermediate Harvest (IMH) could be seen. Plants from stations 3 and 5 produced statistically larger plants (LEN) than those from stations 1 and 4 (Table 20).

Table 20: *Carex arenaria* parameters measured throughout the season 2021

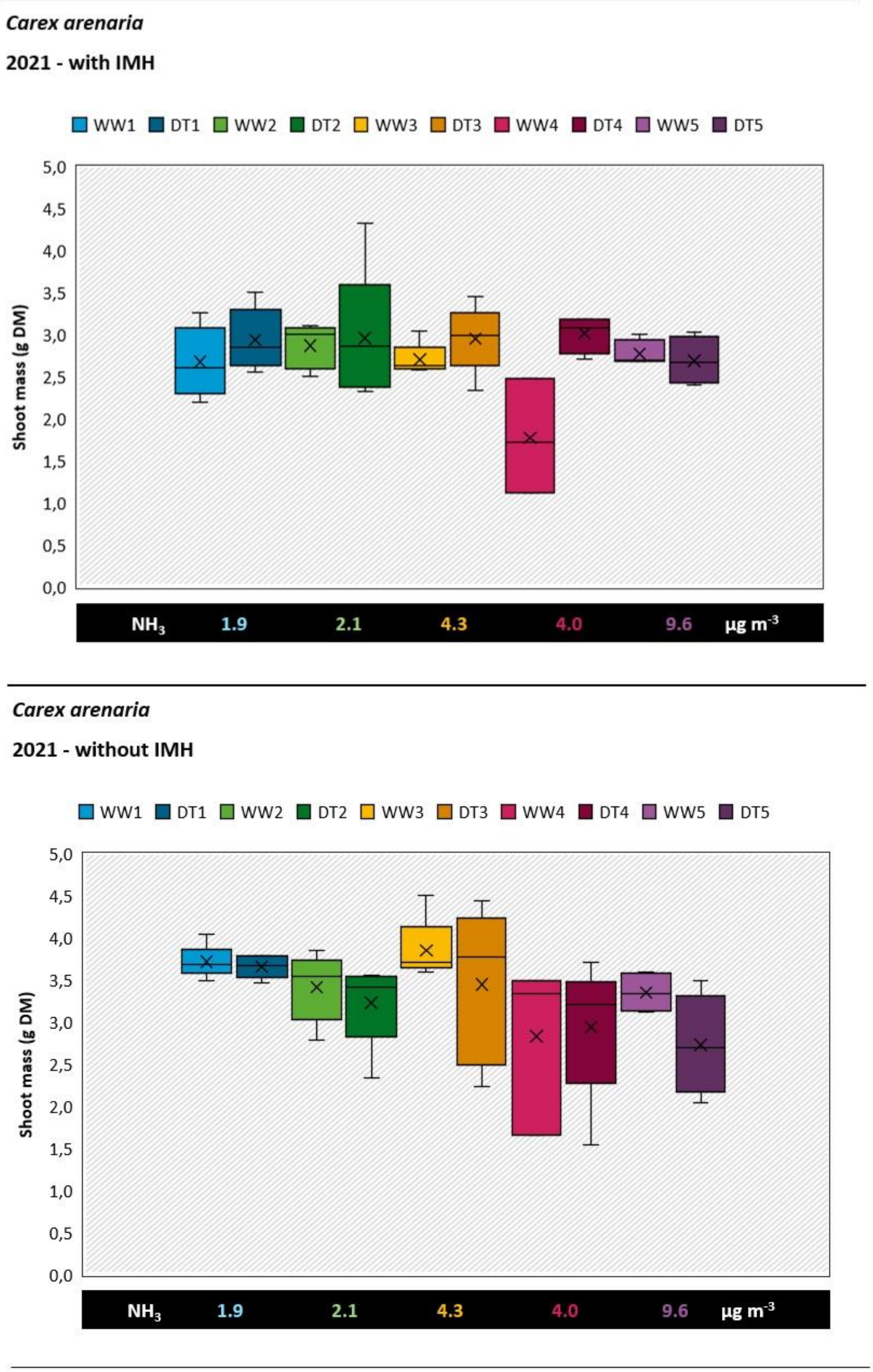
"HEI"= Length [cm]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Add-ons -1 and -2 indicate the belonging of the sets 1 (with intermediate harvest (IMH)) and 2 (without IMH). Numbers 1 to 5 indicate the ammonia treatments; "WW"= Well watered in 2020; "DT"= Dry treatment from 2020 (-33% watering). Different letters within one column indicate statistically significant differences according to multiple comparisons using Tukey tests. In the rows underneath, the differences between certain treatments are given, e.g. "HEI1" is significantly higher in treatments 3 and 5 as in treatment 1.

		Parameter		
NH_3	Water	HEI1	SHOOT-1	SHOOT-2
1	WW	16.1±1.7 a	2.7±0.4 ab	3.7±0.2 a
	DT	16.9±1.2 ab	2.9±0.3 a	3.7±0.1 a
2	WW	17.3±2.1 ab	2.9±0.2 a	3.4±0.4 a
	DT	17.1±1.6 ab	3.0±0.7 a	3.2±0.5 a
3	WW	19.2±1.0 b	2.7±0.2 ab	3.9±0.3 a
	DT	18.3±1.9 ab	3.0±0.4 a	3.4±0.8 a
4	WW	15.1±1.8 a	1.8±0.6 b	2.8±0.8 a
	DT	17.4±1.4 ab	3.0±0.2 a	2.9±0.7 a
5	WW	18.1±2.9 ab	2.8±0.1 ab	3.3±0.2 a
	DT	19.0±4.2 b	2.7±0.3 ab	2.7±0.5 a
H_2O				
NH_3		*3,5>1;3,5>4		*1>4
H_2OxNH_3			*	

The shoot mass differed between plants with (SHOOT-1) and without intermediate harvest (CUT, i.e. SHOOT-2) in 2020. Plants without the additional harvest produced more biomass than the others (Figure 25) suggesting that more resources were available in these plants for regrowth. For plants with IMH an interaction of water and ammonia treatments could be seen. Plants without IMH produced more biomass at station 1 compared to station 4. Apart from that, no clear effects were detected. The absence of significant differences between the WW and DT plants suggests that this drought tolerant species readily recovers from drought stress acting in the previous season.

Figure 25: Total shoot mass of *Carex arenaria* with/without previous intermediate harvest

Shoot mass was determined on 6 October 2021, after the final harvest. Colour codes indicate the arrangement of station numbers after ammonia concentration levels. Left: Plants with intermediate harvest in 2020, right: Plants without intermediate harvest in 2020. Numbers on the x-axis indicate the respective mean ammonia concentrations (µg m^{-3}).



Source: Own illustration, University of Hohenheim.

3.3.2.2.1.7 Dianthus deltoides

Dianthus plants were the last to be transferred to ULI on 30 June 2021 until 25 August 2021 (for 56 days only). The mean NH_3 concentrations for stations 1-5 were 1.7, 2.1, 3.1, 4.2 and 10.4 µg m^{-3}. Plant length (HEI) and shoot dry weight (SHOOT) were determined at the final harvest. Interestingly, plant length was significantly affected by last year's drought treatment, but in a rather unexpected way.

Plants from the dry treatment were taller than the WW plants (Table 21). The shoot mass, however, was neither affected by the watering nor by the ammonia treatments, but we must hint to the fact that the total exposure time was rather short and the shoot mass small in comparison to the other plant species (Figure 26).

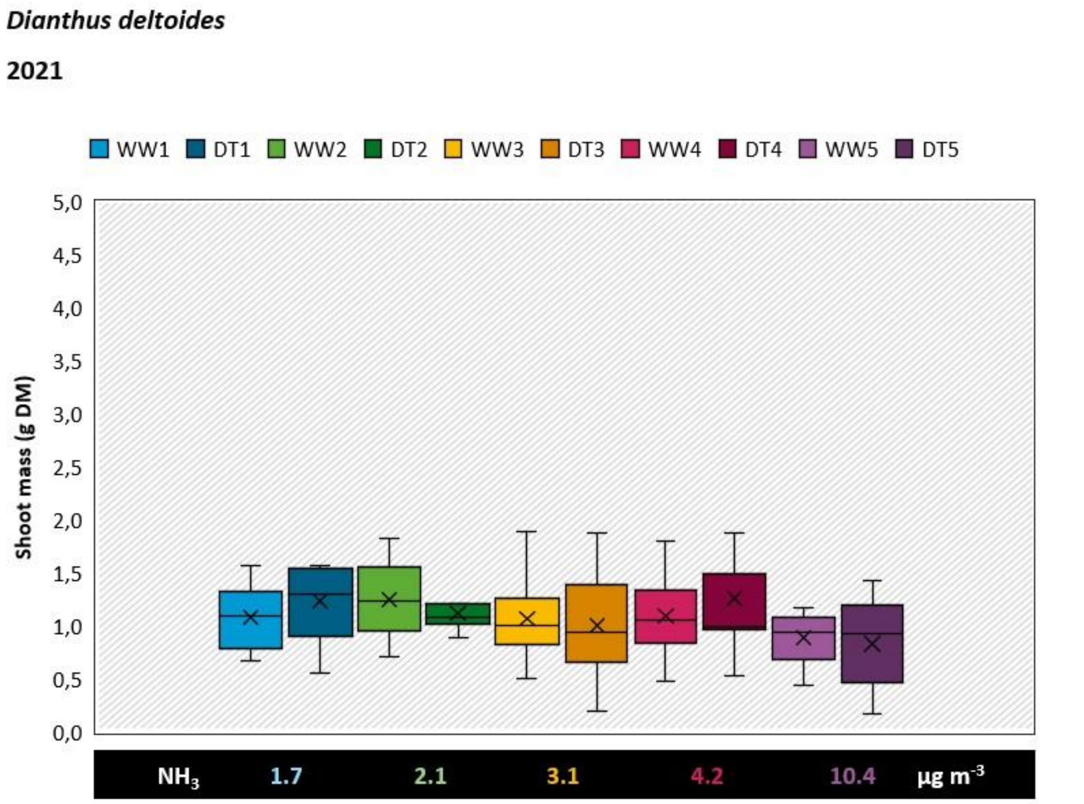
Table 21: *Dianthus deltoides* parameters measured throughout the season 2021

"HEI"= Length [cm]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments; "WW"= Well watered in 2020; "DT"= Dry treatment from 2020 (-33% watering). Different letters within one column indicate statistically significant differences between treatments using Tukey tests.

Treatments NH_3	Water	Parameter HEI1	SHOOT-1	SHOOT-2
1	WW	13.9±4.2 ab	1.2±0.3 a	0.9±0.2 a
	DW	22.0±5.6 a	1.2±0.4 a	1.3±0.3 a
2	WW	15.0±4.8 ab	1.2±0.4 a	1.3±0.3 a
	DW	19.5±5.0 ab	1.1±0.2 a	1.1±0.1 a
3	WW	14.9±5.5 ab	1.0±0.4 a	1.1±0.3 a
	DW	17.3±7.3 ab	0.7±0.3 a	1.3±0.4 a
4	WW	12.1±4.9 b	1.1±0.4 a	1.1±0.4 a
	DW	20.9±3.0 a	0.9±0.2 a	1.7±0.8 a
5	WW	13.2±6.4 b	1.0±0.1 a	0.7±0.2 a
	DW	19.2±5.6 ab	1.1±0.3 a	0.6±0.4 a
H_2O		*1<2		
NH_3				
H_2OxNH_3				

Figure 26: Total shoot mass of *Dianthus deltoides*

Shoot mass was determined on 25 August 2021, after the final harvest. Colour codes indicate the arrangement of station numbers after ammonia concentration levels. Note: The plants were not cut twice in 2020. Numbers on the x-axis indicate the respective mean ammonia concentrations (µg m^{-3}).



Source: Own illustration, University of Hohenheim.

3.3.2.2.2 Plants only exposed in 2021

Species added to the field gradient study involved five annual species with ten replicates per ammonia treatment and one perennial species with 20 replicates per ammonia treatment because no drought could be implemented. In the following sub-chapters, detailed information about the performance of each species are given.

3.3.2.2.2.1 Dianthus gratianopolitanus

The species was the only perennial species to be added to the experiment in 2021. We wanted to include the species due to its very low N tolerance (N=1) and the threat of extinction it is facing. We initially planned to perform a drought stress like for the other perennial species, but this could not be realized due the moist conditions in 2021. Therefore, not only 10 but 20 replicates per treatment were available to address potential NH_3 responses.

Pots were exposed at the ULI stations from 12 May until 18 August, for 98 days. Mean ammonia concentrations during that time were 1.9, 2.1, 3.4, 4.2 and 10.2 µg m^{-3}. The flower number was counted four times in total because plants had been producing many flowers over the season and plants flowered for several weeks. At no time, a significant difference between the five stations could be seen (Table 22).

With regard to the plant length, a significant difference between station 3 and 1 could be observed at the second measuring date. The shoot biomass did not differ significantly between the five stations and no obvious ammonia effects were seen (Figure 27). Plants were neither negatively, nor positively affected by the higher ammonia concentrations. The reason for their threat of extinction therefore seems to lie rather in the advantage other species can gain from higher ammonia inputs than in the direct adverse effects of ammonia or N-input in general on the plants.

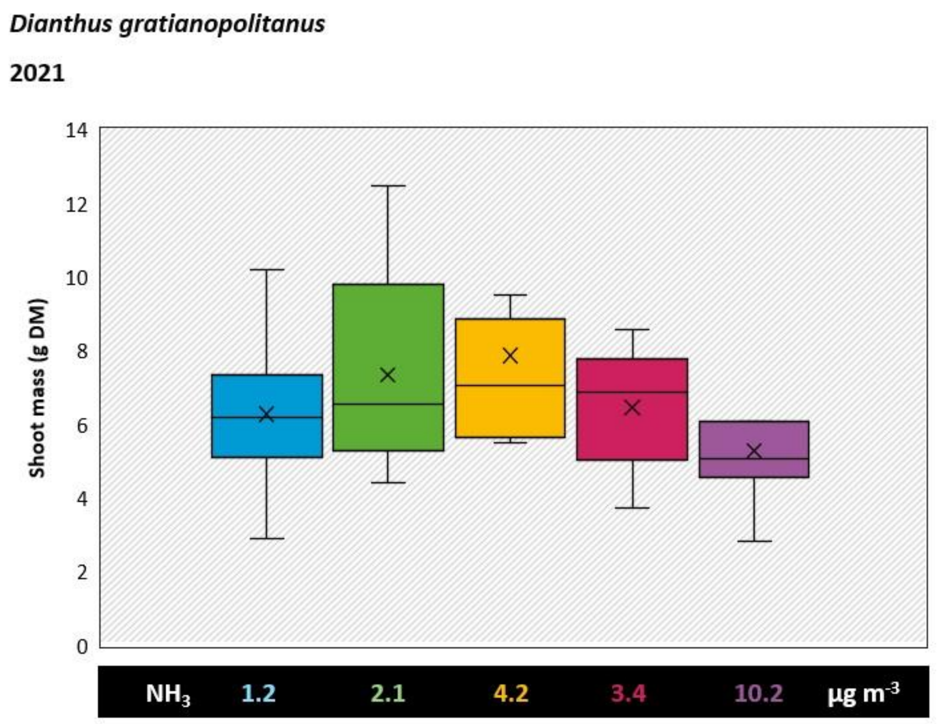
Table 22: *Dianthus gratianopolitanus* parameters measured throughout the season 2021

"FLON"= Flower Number; "HEI"=Length [cm]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments. Different letters within one column indicate statistical differences between treatments using Tukey tests.

NH_3	Parameter FLON1	FLON2	FLON3	FLON4	HEI1	HEI2	SHOOT
1	0.3±0.7 a	0.5±0.8 a	24.2±8.4 a	23.6±8.5 a	18.9±3.5 a	22.4±3.0 a	6.3±1.7 a
2	0.1±0.3 a	1.4±1.5 a	27.1±11.5 a	26.1±10.9 a	19.9±4.0 a	24.2±3.6 ab	7.3±2.4 a
3	0.2±0.5 a	1.2±2.2 a	28.5±12.7 a	27.1±12.0 a	19.1±4.0 a	27.3±4.8 b	7.3±2.2 a
4	0.3±0.6 a	1.7±2.9 a	22.6±9.6 a	21.1±6.9 a	18.7±3.7 a	24.3±4.0 ab	6.5±1.6 a
5	0.4±0.8 a	1.2±2.4 a	21.9±7.8 a	23.1±6.6 a	18.5±4.1 a	24.5±5.1 ab	5.8±1.5 a
NH_3						* 3>1	

Figure 27: Total shoot mass of *Dianthus gratianopolitanus*

Shoot mass was determined on 18 August 2021. Colour codes indicate the arrangement of station numbers after ammonia concentration levels. Numbers indicate the ammonia treatments (1-5) with realized mean concentrations (µg m^{-3}).



Source: Own illustration, University of Hohenheim.

3.3.2.2.2.2 Erodium cicutarium

Erodium was the first annual species to be exposed from 18 March until 30 June 2021, for 104 days in total. The mean NH_3 concentrations during that time were 1.8, 2.0, 5.2, 3.6 and 9.7 µg m^{-3} for the five stations, respectively. Plant length (HEI) and flower number (FLON) were determined once before the harvest. Then, plant length, flower number and the total aboveground biomass were analysed. The first flower measurement (9 June 2021) revealed that plants from the highest NH_3 treatment produced significantly less flowers than the lowest treatment. Only three weeks later, during the harvest, this effect had been compensated and no more difference was visible (Table 23). The earlier effects observed before can hint to differences in the phenological development due to ammonia. However, such responses have never been reported for the gas, but e.g. for the air pollutant ozone.

The first length measurements took place on the same date as the first flower measurement, but plants from all stations grew evenly. Until the final harvest, plants from station 5 had been growing larger than the rest of the plants, mainly than plants from station 1 and 4. With regard to the shoot mass, plants from station 5 also produced more biomass, especially in comparison to station 2 (Figure 28). The plants reached a length of up to 10 cm only, whereas plants in nature can reach up to 60 cm. The Ellenberg N value of the annual is indifferent which indicates that plants can grow in N poor as well as in N rich environments.

We can see that for *Erodium cicutarium* the growth parameters length and shoot mass were higher under the highest ammonia concentration (station 5) and suggest that this can be related to the higher nutrient input in form of ammonia. The negative effect on the flower number in the beginning of the plant development did not persist for long.

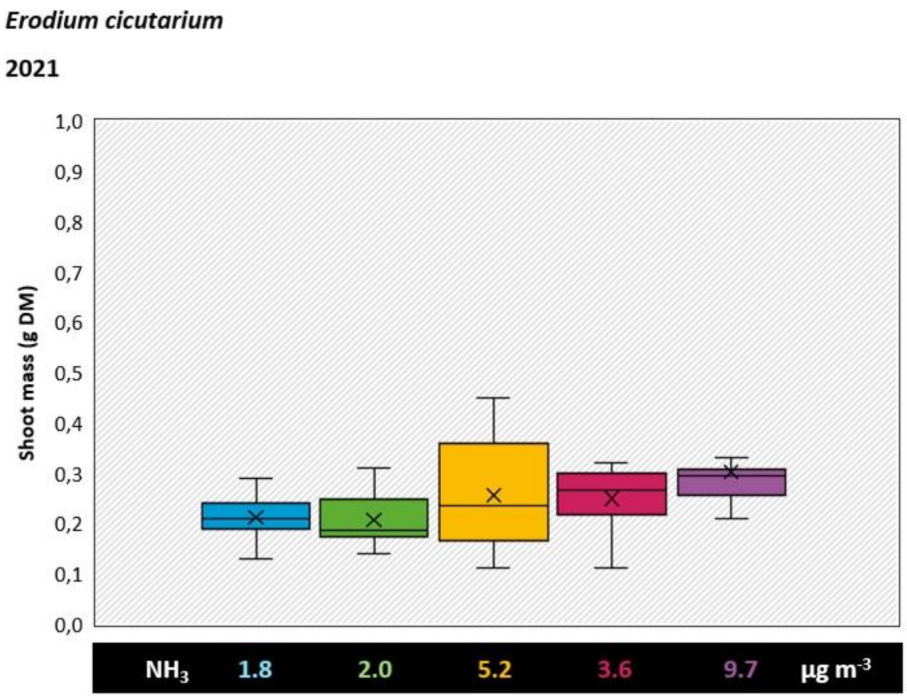
Table 23: *Erodium cicutarium* parameters measured throughout the season 2021

"FLON"= Flower Number; "HEI"=Length [cm]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments. Different letters within one column indicate statistical differences between the treatments using Tukey tests.

Treatment	**Parameter**				
NH_3	FLON1	FLON2	HEI1	HEI2	SHOOT
1	3.2±3.1a	19.1±4.7 a	3.7±1.6 a	7.9±1.2a	0.21±0.0 ab
2	2.1±2.7 ab	18.4±4.6 a	3.1±1.1 a	8.3±1.2 ab	0.21±0.1 a
3	0.5±0.9 ab	18.7±4.7 a	3.7±1.8 a	8.6±1.3 ab	0.26±0.1 ab
4	2.4±2.6 ab	21.7±5.1 a	2.9±0.9 a	8.0±0.9 a	0.25±0.1 ab
5	0.2±0.6 b	21.7±4.2 a	2.9±0.8 a	9.8±1.0 b	0.30±0.1 b
NH_3	* 1>5			* 5>1,4	*5>2

Figure 28: Total shoot mass of *Erodium cicutarium*

Shoot mass was determined on 30 June 2021. Colour codes indicate the arrangement of station numbers after ammonia concentration levels. Numbers indicate the ammonia treatments (1-5) with realized mean concentrations (µg m-3).



Source: Own illustration, University of Hohenheim.

3.3.2.2.2.3 Bromus hordeaceus

Bromus plants were cultivated in groups of three individuals per pot and were exposed from 6 May until 16 September (133 days) at ULI. The mean ammonia concentrations during that time were 1.8, 2.0, 3.7, 3.9 and 9.6 µg m-3. Plants did not grow much and no flowers were produced. We assumed that three individuals could not deal with the low amount of nutrients per pot. Therefore, only at the final harvests the shoot mass was determined and distinguished between green and senescent fractions. In all three fractions, it became visible that plants from station 5 produced the highest biomass (Table 24, Figure 29).

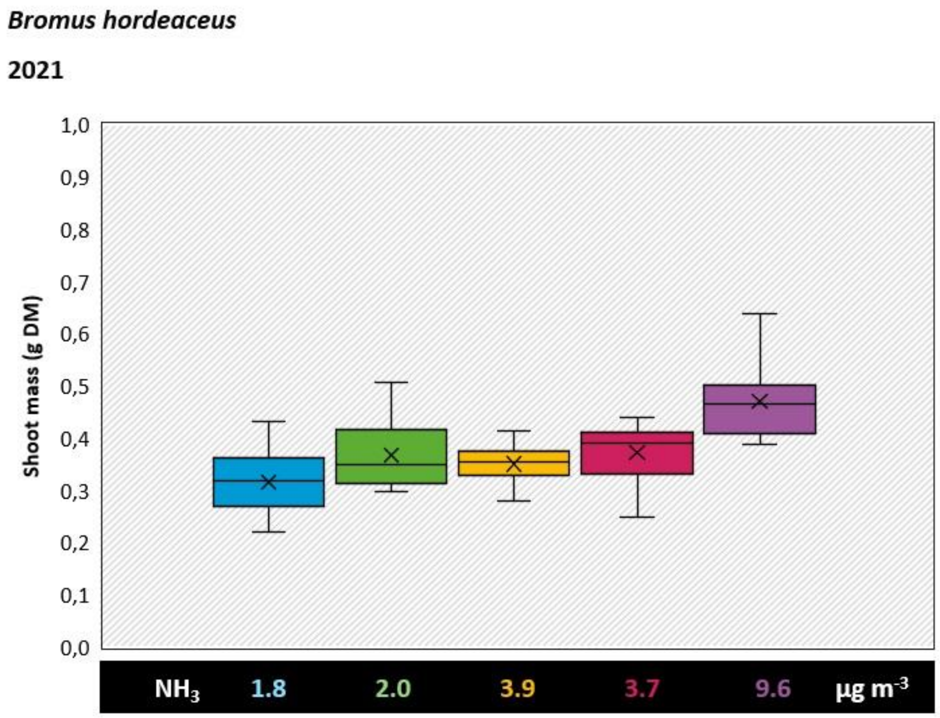
Table 24: *Bromus hordeaceus* parameters measured throughout the season 2021

"SEN"= Senescent Biomass [g]; "GREEN"=Green Biomass [g]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments. Different letters within one column indicate statistical difference according to Tukey HSD tests.

Treatment NH_3	Parameter SEN	GREEN	SHOOT
1	0.22±0.04 a	0.09±0.02 a	0.31±0.06 a
2	0.27±0.05 a	0.09±0.02 a	0.37±0.06 a
3	0.27±0.03 a	0.08±0.02 a	0.35±0.04 a
4	0.28±0.04 ab	0.10±0.02 a	0.37±0.05 a
5	0.33±0.05 b	0.14±0.02 b	0.47±0.07 b
NH_3	* 5>1,2,3	* 5>1,2,3,4	* 5>1,2,3,4

Figure 29: Total shoot mass of *Bromus hordeaceus*

Shoot mass was determined on 16 September 2021. Colour codes indicate the arrangement of station numbers after ammonia levels. Numbers indicate ammonia treatments 1-5 with realized mean concentrations (µg m^{-3}).



Source: Own illustration, University of Hohenheim.

Although this species can generally cope with nitrogen-poor soils (Ellenberg N=3) and although the highest ammonia concentration improved its growth, *Bromus* could not complete its full life cycle under the given conditions. It may well be that the grass is a facultative biannual and would have only flowered in the second season. Anyway, the species growth was enhanced through ammonia even at a low concentration of around 10 µg m^{-3}. In the highest NH_3 treatment, plants produced on average 50% more shoot mass than at a concentration of 1.8 µg m^{-3}.

3.3.2.2.2.4 Iberis amara

Iberis plants were introduced into the field gradient study on 12 May 2021 and exposed for 70 days until 21 July. The mean ammonia concentrations during that time were 2.0, 2.2, 3.5, 4.2 and 10 µg m^{-3}. Plant length was measured twice before the final harvest (HEI1 and HEI2) and on the final harvest date, flower number (FLON1), length (HEI3) and shoot mass (SHOOT) were determined.
Regarding the plant length in the beginning of the season (20 May and 9 June), no differences between the ammonia treatments could be observed, but plants had only been exposed for some weeks until then. Afterwards, plants from station 5 were significantly taller than those from the other stations (Table 25). Additionally, the flower number was higher at station 5 compared to station 3 and the total shoot mass was also highest at station 5 (statistically significant when compared to station 2 and 3, Figure 30).

We can state that also *Iberis* showed enhanced growth in relation to the highest ammonia concentrations during the season, but only after having been exposed for a certain time. This makes sense because the uptake and metabolism of ammonia take some time and will be reinforced by the stronger shoot growth and larger surface area.

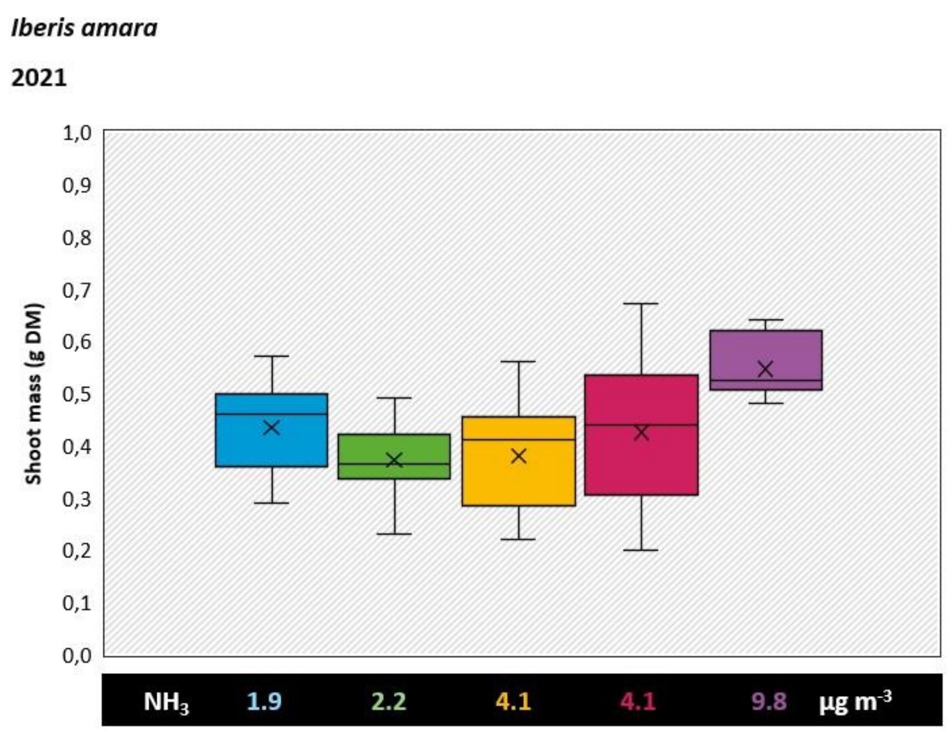
Table 25: *Iberis amara* parameters measured throughout the season 2021

"FLON"= Flower Number; "HEI"= Plant height [cm] at three dates; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments. Different letters within one column indicate statistical differences between the treatments as checked by Tukey tests, e.g. plants from treatment 5 had significantly more flowers than those in treatment 3.

Treatment	Parameter				
NH_3	FLON1	HEI1	HEI2	HEI3	SHOOT
1	29.2±8.6 ab	5.0±0.5 a	11.2±1.9 a	30.4±4.0 a	0.43±0.09 ab
2	29.3±8.4 ab	4.8±0.8 a	11.2±2.0 a	28.5±3.7 a	0.37±0.07 a
3	23.8±6.9 a	5.3±0.5 a	13.4±4.2 a	30.3±5.7 a	0.38±0.10 a
4	33.0±13.2 ab	5.2±0.8 a	12.1±2.6 a	29.3±5.6 a	0.43±0.13 ab
5	40.0±5.1 b	5.4±0.5 a	14.6±2.0 a	36.8±3.1 b	0.55±0.06 b
NH_3	* 5>3			* 5>1,2,3,4	*5>2,3

Figure 30: Total shoot mass of *Iberis amara*

Shoot mass was determined on 21 July 2021. Colour codes indicate the arrangement of station numbers after ammonia levels. Numbers indicate the ammonia treatments (1-5) with realized mean concentrations (µg m^{-3}).



Source: Own illustration, University of Hohenheim.

3.3.2.2.2.5 *Trifolium arvense*

Trifolium plants were exposed from 24 June 2021 until 22 September 2021, for 90 days in total. The mean ammonia concentrations during that time were 1.9, 2.2, 4.1, 4.1 and 9.8 µg m^{-3}. The first measurement taken was the number of flowers on 11 August when no statistical differences between plants from the five stations could be seen. At the harvest date (22 September 2021) flowers were counted again and it turned out that plants from station 5 had been producing less flowers than plants from the other stations with a significant difference to those from station 3 (Table 26). This pattern could also be observed for the flower dry weight as well as the flower's share to the whole shoot mass. This is interesting because *Trifolium* was the only plant species that reacted negatively to the highest NH_3 concentration, but only the parameters concerning the reproductive organs were affected.

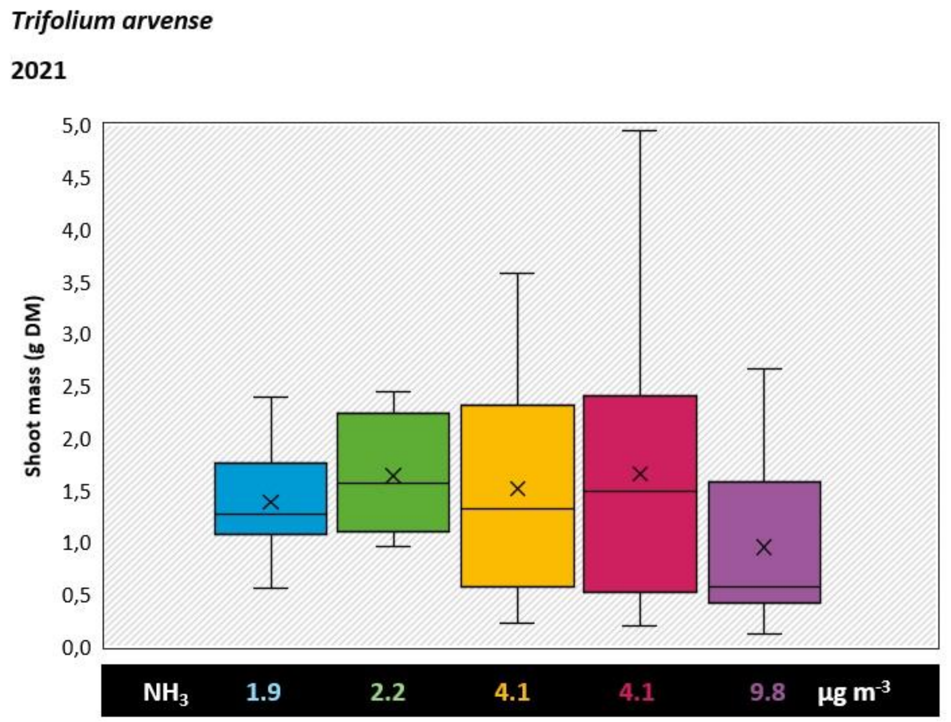
Table 26: *Trifolium arvense* parameters measured throughout the season 2021

"FLON"= Flower Number; "HEI"=Length [cm]; "FLOWDW"=Flower Dry Weight [g]; "STEMDW"= Stem Dry Weight [g]; "FLOW%"=Flower proportion [%]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments. Different letters within one column indicate statistical differences between the ammonia treatments according to Tukey tests, e.g. plants from treatment 5 had significantly lower flower numbers than those from station 3

Treatment NH_3	Parameter FLON1	FLON2	FLOWDW	FLOW%	HEI1	STEMDW	SHOOT
1	1.1±2.3 a	16.4±6.3 ab	0.42±0.2 ab	36.9±12.1 ab	26.0±6.5 a	0.97±0.3 a	1.39±0.5 a
2	0.9±2.7 a	14.9±8.0 ab	0.26±0.2 a	32.2±13.3 ab	29.0±6.9 a	1.38±0.5 a	1.63±0.5 a
3	5.5±5.5 a	25.9±11.0 a	0.62±0.3 b	47.6±9.6 a	26.2±6.1 a	0.89±0.7 a	1.51±1.0 a
4	5.8±12.2 a	17.9±13.9 ab	0.39±0.4 ab	35.2±20.9 ab	22.3±9.7 a	1.26±1.2 a	1.65±1.4 a
5	0.0±0.0 a	9.0±8.6 b	0.16±0.2 a	21.1±14.0 b	22.6±9.9 a	0.79±0.7 a	0.95±0.8 a
NH_3		*5<3	*3>2,5	* 5<3			

Figure 31 Total shoot mass of *Trifolium arvense*

Shoot mass was determined on 22 September 2021. Colour codes indicate the arrangement of station numbers after ammonia levels. Numbers indicate the ammonia treatments (1-5) with realized mean concentrations (µg m^{-3}).



Source: Own illustration, University of Hohenheim.

Plant length, stem weight and the total shoot weight (Figure 31) were, however, not significantly influenced by the different concentrations. A trend towards a lower shoot biomass could, however, be observed. A lower flower, i.e. seed, and shoot production would directly negatively affect *Trifolium arvense*'s competitive capacity. This might be a hint that legumes may respond negatively to airborne ammonia, but this needs to be further studied as we have only used one leguminous species in our experiment and cannot evaluate the results since literature on this topic is lacking. Like mentioned above, other air pollutants like ozone may have effects on reproduction. Direct effects may turn up if air pollutants interact with e.g. the formation of pollen.

3.3.2.2.2.6 Legousia speculum-veneris

Legousia plants were exposed for only 36 days, from 9 June 2021 until 15 July. The life cycle of this annual is rapidly finished and the plants flowered readily in the field. The mean ammonia concentrations during that time were 2.8, 3.2, 4.6, 4.8 and 11.2 µg m^{-3}.

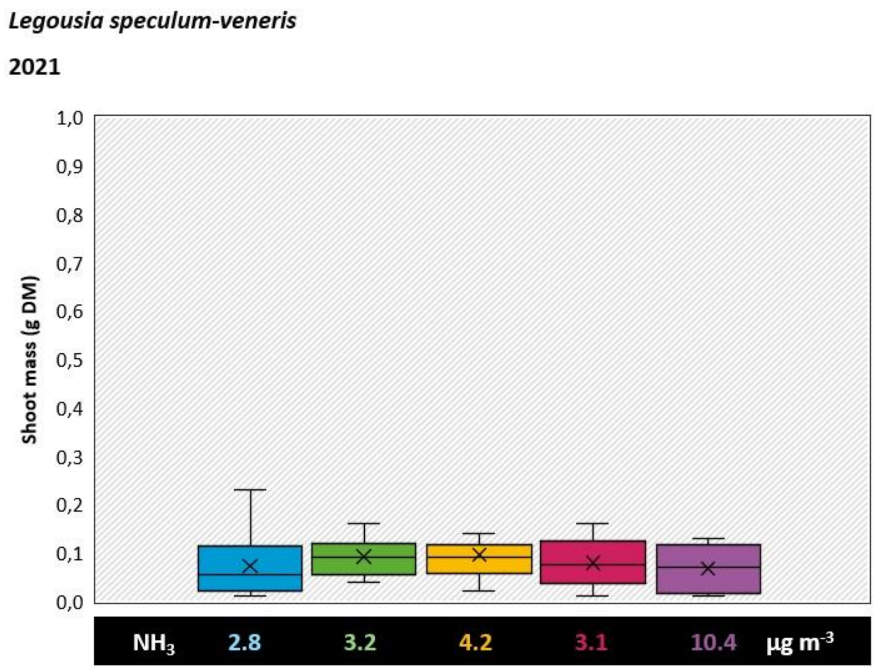
Table 27: *Legousia speculum-veneris* parameters measured throughout the season 2021

"FLON"= Flower Number; "HEI"=Length [cm]; "SHOOT"=Total Shoot Biomass [g] ± standard deviation. Numbers 1 to 5 indicate the ammonia treatments. Different letters within one column indicate statistical differences according to multiple comparisons using Tukey tests.

Treatment NH_3	Parameter FLON	HEI	SHOOT
1	6.1±3.0 a	9.5±3.8 a	0.07±0.06 a
2	7.4±2.8 a	11.5±1.7 a	0.09±0.04 a
3	6.9±3.7 a	11.4±2.2 a	0.10±0.05 a
4	7.6±3.8 a	9.6±1.6 a	0.08±0.05 a
5	5.9±3.0 a	12.0±2.6 a	0.07±0.04 a
NH_3			

Figure 32: Total shoot mass of *Legousia speculum-veneris*

Shoot mass was determined on 22 September 2021. Colour codes indicate the arrangement of station numbers after ammonia levels. Numbers indicate ammonia treatments 1-5 with realized mean concentrations (µg m^{-3}).



Source: Own illustration, University of Hohenheim.

We determined the flower number (FLON), plant length (HEI) and shoot biomass (SHOOT) at the final harvest and for all parameters, no significant differences between the stations, respectively ammonia concentrations, could be seen (Table 27, Figure 32). It may well be that ammonia responses in annuals with very short life cycles are generally not pronounced, at least at those low levels realized in present experiment.

3.3.2.2.3 Summary of the plant responses

A summary of all results of the field gradient study is given in Table 28. For those plants which were already exposed in the chamber study, we can state that the intermediate harvests (IMH), which were performed in 2020 and simulated mowing, in several ways seems to have influenced the behaviour and response of plants in the second year during the field gradient study. Firstly, IMH restricted a higher shoot production in the following season. At the same time, ammonia effects were less likely to occur in plants, which had been harvested twice in the preceding season. Next, nearly all IMH-plants produced significantly less biomass. Ammonia effects independent from the drought were only detected in plants without intermediate harvest. We explain this with the fact that the additional removal of plant biomass led to a pronounced removal of nutrients. Replenishing nutrients would have been crucial for a higher biomass production in the exhausted soil. In *Dianthus deltoides*, no ammonia effects were observed, but plants had only been exposed for 56 days, the shortest period compared to the other perennial species from last year.

With regard to the water availability, the shoot mass in 2021 in most species was not affected by the drought exerted in the previous season. In *Molinia*, however, the watering affected the response to NH_3 significantly: Plants with intermediate harvest only reacted to the different concentrations when they had been kept in the DT in 2020. *Antennaria* and *Carex* plants also showed a coupling of water and ammonia effects, but this was not as clearly interpretable as in *Molinia*.

Regarding the annual species, three out of five species showed enhanced growth at the highest ammonia concentrations with reflected in their shoot biomass. One species, i.e. *Trifolium arvense* tended to be negatively affected by the highest concentration, but the effect was not statistically significant. *Legousia* did not react to the different concentrations, but we think that the short exposure time and growth cycle probably plays a major role here. On the other hand, the perennial *Dianthus gratianopolitanus* was exposed longer but did not show differences in growth as well. We therefore conclude that not only the exposure time, the length of the growth cycle but also the identity (taxonomy) of a species needs to be respected. For all species, the rather high standard deviations need to be considered. This was the case both in the species with 10 and even 20 replicates. In the beginning, we hypothesised that fast growing species would sooner experience the need to replenish nutrients and would be ready to take up ammonia from the air. When looking at the results, we observed that this was rather not the case. The annual species which did not react significantly to the different concentrations were *Trifolium* that produced the highest biomass among the annual species and *Legousia* that produced the lowest biomass. The three species reacting to the highest ammonia concentrations with higher shoot production were in between with regard to their vigour. The species that produced most shoot biomass, but did not react to the ammonia concentrations was *Dianthus gratianopolitanus*, but this could relate to the fact that there were still ample nutrients in the soil so that plants were not N limited and were not dependent on ammonia-N from the air. The following table gives a concise overview of all results (Table 28).

Table 28 Overview of the results from the season 2021

"CUT" or "UNCUT" indicates whether plants had been subjected to an intermediate harvest in the preceding season or not. "✓" indicates an effect and numbers indicate which treatments were significantly different from each others.

Plant species from 2020	*Antennaria*	*Carex*	*Dianthus d.*	*Koeleria*	*Molinia*	*Pulsatilla*
Exposure time (days)	104	104	56	202	124	202
Shoot mass						
CUT						
UNCUT		✓		✓	✓	✓
Shoot ammonia effect						
CUT						
UNCUT		✓1>4		✓1>2	✓3,4,5>1; 5>2	
Shoot water effect						
CUT					✓	
UNCUT						
Shoot interaction effect						
CUT	✓	✓				
UNCUT						
Plant species from 2021	*Dianthus g.*	*Bromus*	*Erodium*	*Iberis*	*Legousia*	*Trifolium*
Exposure time (days)	98	133	104	70	36	90
Shoot ammonia effect		✓5>1,2,3,4	✓5>2	✓5>2,3		

3.4 Isotopic analyses and N concentrations

In order to be able to address whether and how much of the nitrogen present in the plants was taken up from atmospheric ammonia, we used an N dilution approach and determined the ^{15}N signatures and N concentrations in the shoot mass of two species, namely the grass *Molinia*, that had been reported to show positive growth responses under eutrophication and *Arnica*, being a rare species in nutrient poor surroundings. With the dilution of the heavier isotope supplied in the beginning via a fertilisation over time by the lighter ^{14}N isotope present in the ammonia, the initially elevated $\delta^{15}N$ (delta, i.e. the ^{15}N proportion as related to the $^{15}N/^{14}N$ ratio in a sample as compared to the air standard, in per mille) should decrease over time.

While in the two populations of *Arnica montana*, we determined ^{15}N and nitrogen concentrations in a total of eight fractions (five in 2020 and three in 2021) of senescent leaves that were continuously collected throughout the whole experiment, in *Molinia caerulea* we only used the shoot and root mass that was collected in the very end of the field experiment, respectively the second year.

In order to keep the complex processing of samples and costs limited, we focused on these two important species only and in *Arnica* combined different fractions of senescent leaves. In the fumigation study, in the year 2020, the five senescent fractions were collected on 8th April, 30th July, 28th August, 8th October and on 15th December. The combination of fractions SEN1 and SEN2 represents the early to mid-season (April-July), fractions SEN3 and SEN4 the late-season (July to October) and fraction SEN5 the winter. We also analysed three more senescent fractions from the second year, but in these samples the evaluation of results could not be based on the same groups (numbers of items in a sub-treatment) since many individuals died throughout the experiment.

Arnica montana

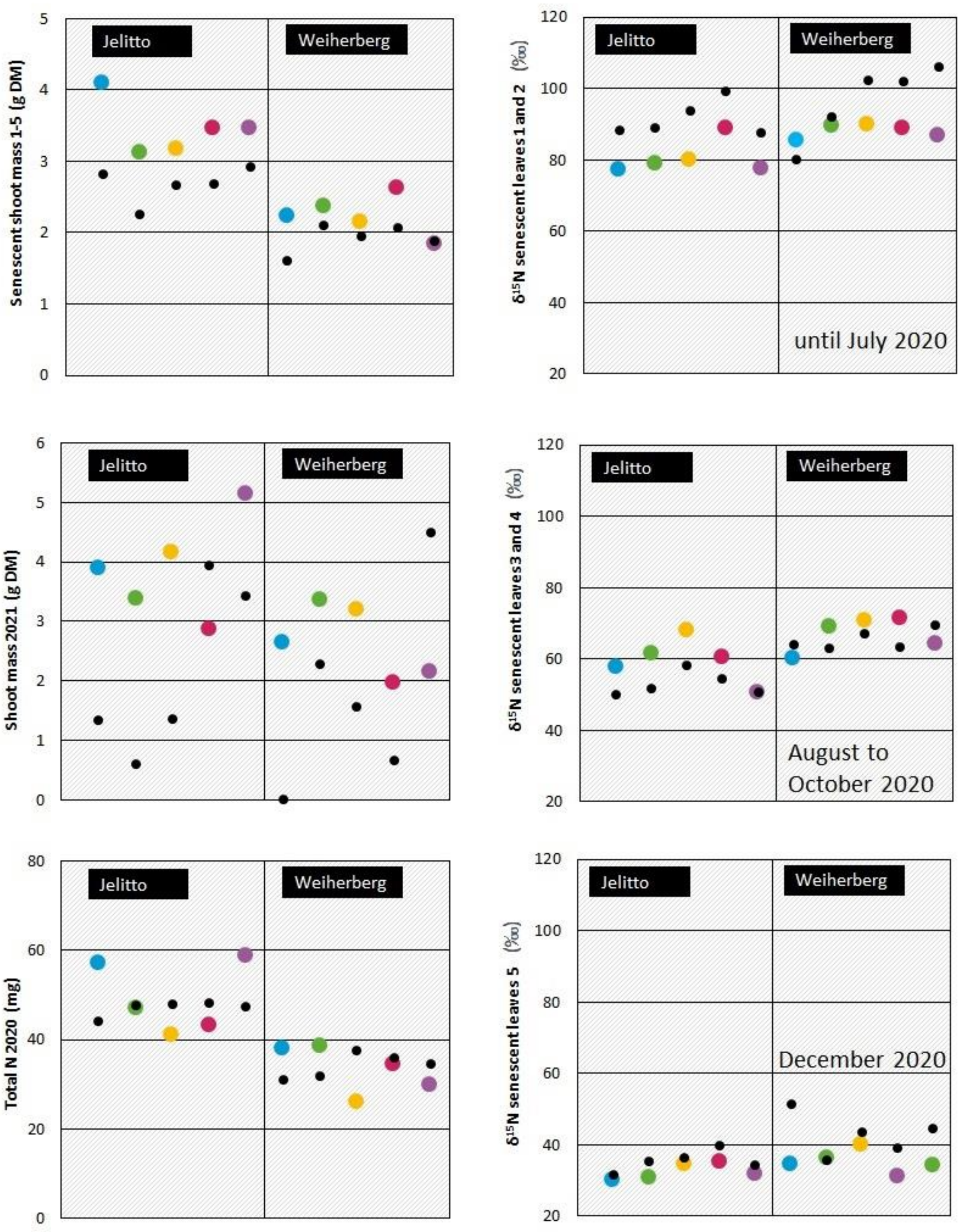
Figure 33 shows the results from the shoot mass and the Isotope-ratio mass spectrometry (IRMS) analyses from senescent leaves of *Arnica montana.* While the plants from the dry treatment (DT) showed lower growth than the well-watered (WW) plants in the first year (2020) of the fumigation study, the effects of the water supply in the preceding season were not clear any more in the field gradient study. Due to rather moist conditions, we were not able to realize a DT in the second year. Since many plants had died during the experiment in the first year, we cannot state whether differences between the ammonia treatments were significant in the next season. In general, shoot mass and the total mass of nitrogen present in the shoot, were not different between the treatments and did not change with the increase of ammonia concentrations from 4 to 17.6 µg m^{-3}.

Larger differences in terms of growth and N accumulation in the shoot than between ammonia treatments were seen between the two cultivars. The commercial seed origin "Jelitto" was overall more productive than the wild "Weiherberg" population in both years. We also noted that more individuals had died in the wild mountain origin, showing that it is probably less resilient than the "improved" cultivar. However, we do not have any information on where the original population of the Jelitto cultivar came from.

With regard to the ^{15}N analyses in the different fractions of the species, we also observed differences between the two populations as well as modifications due to the supply of water.

Figure 33: Total shoot mass of two *Arnica montana* populations in the years 2020 and 2021, total shoot N contents and ^{15}N isotopic ratios in the senescent leaves

Black dots refer to dry treatment (DT) and coloured dots to well-watered (WW) plants. Colours refer to the five ammonia treatments using the same scheme that is used throughout the report. Sub-panels differentiate between the plants from the "Jelitto" commercial and the "Weiherberg" wild population. Senescent fractions 1 and 2 refer to the first collections of dead leaf material until 30th July 2020, fractions 3 and 4 represent the collections of senescent leaf material until 8th October 2020. and senescent fraction number 5 was collected on 15th December 2020. While on the left side productivity and nitrogen acquisition are shown, the dilution of ^{15}N over time is shown in the three panels on the right side.



Source: Own illustration, University of Hohenheim.

These were much more pronounced in the early season and vanished with the increasing growth of the plants. With the consecutive harvests of senescent material from July to December 2020, we removed more of the heavier N isotope that was "captured" in the dead leaf mass. With the supply of N and the higher proportions of the lighter ^{14}N from the ammonia, a decrease in the δ signatures was observed. A major driver of the isotopic dilution is therefore the development of the senescence process. In the slower growing plants of the DT, we can observe that in the beginning much more of the heavier isotope was still present in the plants that were grown under elevated concentrations of ammonia (treatments 3 to 5). On the other hand, the slightly lower δ signatures in the senescent leaf mass from treatment 5 in the "Jelitto"-origin at all three dates (until 30th July, October 8th and December15th) confirms that more isotopic dilution must have taken place in the plants that were grown under a higher supply of ammonia. While the approach seemed to work, we were not able to quantify how much of the absorbed nitrogen stemmed from the ammonia fumigation. The results only show that at ammonia concentrations of less than 20 µg m^{-3} plants can indeed assimilate N from the atmosphere.

Molinia caerulea

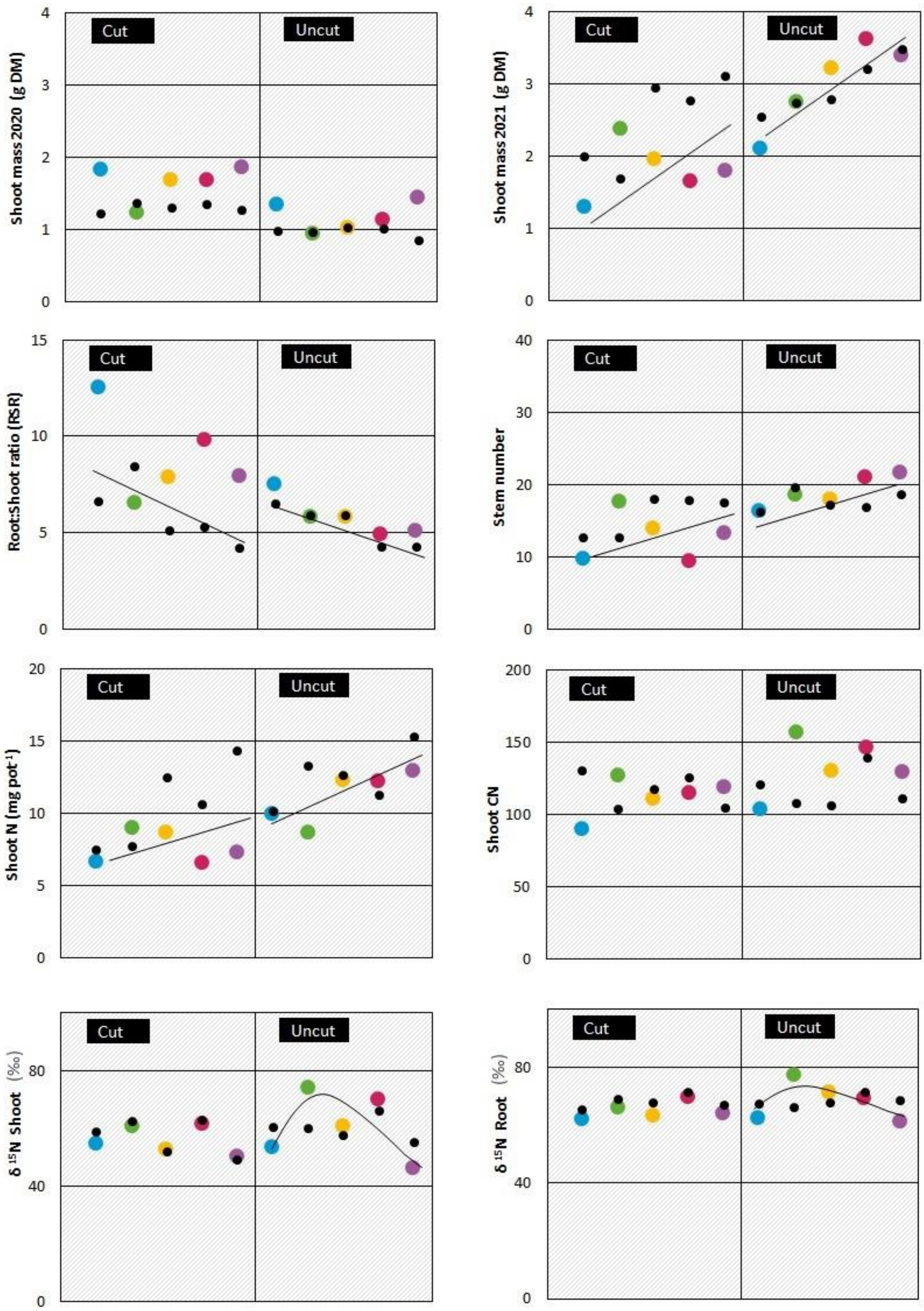
Like the other species, the Purple Moorgrass was also fertilised with ^{15}N labelled fertiliser to be able to trace whether the grass can take up nitrogen from the deposition of the gas ammonia. For *Molinia caerula* we did not test different seed origins, but used clones (ramets) from the species that were subjected to moist and dry conditions and a simulated mowing.

In contrast to *Arnica*, the plants of *Molinia caerulea* did not produce many senescent leaves during the experiment, so that harvests took place only in the end of the season. Senescence in the grass was occurring progressively and was difficult to assess since leaves turned yellowish over a long time. In contrast, in the herbaceous *Arnica montana* leaf shedding occurred within a few days and it was easy to separate dead from alive leaves. Since we could not do that in *Molinia*, we subjected half of the plants to an intermediate harvest in the first year of the experiment to see how the removal of biomass interacted with drought and the supply of N via the ammonia fumigation. After the cut, the plants soon developed new leaves indicating that the grass can very well tolerate the removal of shoots and N and other nutrients contained therein.

Figure 34 shows the results from the shoot mass and IRMS analyses that were obtained from *Molinia caerulea.* Like in *Arnica*, the growth parameters as well as the ^{15}N analyses showed differences between the dry-treated (DT) and the well-watered (WW) plants of this species that is common in both, dry and wet grasslands. However, these responses were modified by the removal of shoot mass during the season. Performing two harvests (Cut) led to a higher total shoot mass in the first year as compared to plants that were only cut in the end of the season (Uncut). The opposite trend was observed in the following season, most likely because previously uncut plants were able to maintain more resources available for the next season. This is also confirmed by the finding that plants from the DT that were subjected to an intermediate and a final harvest in the year before, had higher biomass than those that were previously grown in the WW treatments. The marked interactions between fertilisation and water supply in the performance and growth of *Molinia* were also shown in the field and pot experiments by Falk et al. (2010). N fertilisation was not the sole factor explaining the strong growth increments in encroached heathlands and the allocation patterns (including roots and reproductive structures) proved to be more important than the leaf mass.

Figure 34: Total shoot mass of *Molinia caerulea* in the years 2020 and 2021, RSR, stem numbers, total shoot N contents and ^{15}N isotopic ratios in the shoot and the root

Black dots refer to DS and coloured dots to WW plants in the respective ammonia treatments. Colours refer to the ammonia exposure and the same scheme that is used in the report. For the respective concentrations refer to the tables 7 and 12. Sub-panels differentiate between the plants that were subjected to an intermediate harvest (cut or uncut).



Source: Own illustration, University of Hohenheim.

It may well be that the exposure to a light stress and a forced slower growth led to a conservation of resources in our experiment. Obviously, the nutrient ecology of *Molinia* is important for its long-term existence and performance from year to year. The perennial grass species is known for effectively recycling nutrients and assimilates in the autumn (Aerts & Caluwe 1991; Thornton 1991, Salim et al. 1995; Taylor et al. 2001) and is often said to show a growth stimulation from N deposition and CO_2 enrichment (Franzaring et al. 2008). Indeed, plants exposed in the vicinity of the stable produced more biomass, whereas in the preceding fumigation shoot mass was not significantly stimulated despite the higher ammonia concentrations in the first year.

The rootstock of the hemicryptophyte tussock-forming *Molinia caerulea* constitutes a massive storage organ (see Figure 21), from which assimilates and nutrients can quickly be remobilised in the spring. During the two-year experiment, the rootstocks of the clones grew tremendously despite the limited pot size and the lack of fertilizers. While ramets in the beginning of the experiment in spring 2020 had an average fresh weight of 10 g, the dry weight of rootstocks was 15 g on average two years later, indicating at least a tripling. At the same time, the average shoot mass produced in a year more than doubled from 2020 to 2021, although the soil was not fertilised. Foliar nitrogen concentrations of the grass are generally much lower and its shoot C:N is much higher than in the herbaceous *Arnica*, indicating that the grass has a high nitrogen use efficiency (NUE). While *Molinia* tends to preserve its resources and tolerates drought and poor soil fertility, *Arnica* tends to shed leaves and resources. However, in the field the nutrients of the shed leaves would soon again be made available as indicated by the low C:N ratio of leaf material from *Arnica*.

As stated above, we could observe a higher shoot mass and a higher total N content in shoots of *Molinia* plants that grew close to the stables. This was true in both, the plants that had been cut and uncut in the preceding season and this was also true for both the WW and DT plants. However, we did not observe a clearly linear response along the N gradient, most likely due to the fact that ammonia levels below 5 $\mu g\ m^{-3}$ cannot be well "sensed" by the grass. The most obvious growth gradient was seen in those plants that had been cut twice and which were grown in the DT in the preceding season, indicating that under growth limitation and a mild stress the grass will more strongly respond to N deposition due to the hunger for nutrients.

Interestingly, the $\delta^{15}N$ signatures of shoot mass from plants near the stables was slightly lower indicating that more of the lighter ^{14}N had been absorbed, especially in the moist uncut, i.e. least stressed plants that were able to take up most of the ammonia. These were also the plants with the lowest root-shoot-ratio (RSR), which indicates a "luxurious" shoot growth. Also, the roots from the uncut WW plants featured the lowest ^{15}N signatures close to the stables, although the higher values (>60 ‰) in roots as compared to the shoots indicate that more of the heavy isotope remained in the belowground plant part. Less ^{15}N dilution, i.e. high values of over 70 ‰ were also seen in the shoots of previously uncut leaves far away from the stable (station 2), whereas in cut plants from the same station more ^{15}N dilution had occurred due to the removal of biomass.

3.5 General discussion

Plant ecologists presume that life history, ecological strategy and functional traits of a species determine its responsivity to the input of nutrients including the gaseous dry deposition of ammonia and other N-containing gases. While gases can be taken up via the stomata and via diffusion through the epidermal cuticles, most of the NH_3 deposited to vegetation will be hydrolysed into aqueous NH_4^+, which will be washed off from the leaves and other parts of the shoots. The nutrient-N can then be taken up via the roots, but the deposition and hydrolysis of ammonia will reduce the soil pH creating secondary responses. The acidification per se will trigger cascade effects not only in the soil, but can also lead to e,g. nutrient imbalances in the plants as well as to changes in the acid-base equilibria needed for enzymatic reactions.

Aim of the study was to investigate ammonia responses of diverse taxa. All of them should be relevant for nature protection and we mainly selected species from nutrient poor environments, e.g. acidic and calcareous grasslands. While perennial species should be tested in two experimental seasons, we included five annuals in the second year. The inclusion of other species in the second year was followed since we could not continue with the greenhouse-based fumigation study. The reason was that we were unable to reduce the heat in the experimental compartments and the plants showed an altered phenology. We therefore decided to perform a field gradient study using the same specimens from the first year as well as additional annual species. Growing the plants outdoors showed a normal development so that potential ammonia effects would not be modified by an altered phenology or heat stress.

A concise summary of the results is presented in Table 29 indicating for each of the species whether positive or negative responses were observed. In order to study the growth during the two experimental seasons, we also related the shoot biomass of plants produced in the second season to that one of the first year and derived 2021/2020 biomass ratios. Interestingly, three perennial species produced more biomass in the second season as compared to the first year although no extra fertilisers were supplied. Growth was especially boosted in the grass species *Molinia caerulea* and the sedge *Carex arenaria* as indicated by the biomass ratio 2021/2020 in the last column of the table. Besides the graminoids, the two composites *Antennaria dioica* and *Arnica montana* (both origins) were also able to produce more shoot mass in the second year due to the fact that they produced flowers in the field gradient study.

Averaged over the two years, arnicas from the commercial seed supplier (Jelitto) were the most productive plants with on average 3.06 g dry mass in 2020 and 3.34 g dm in 2021. However, it must be pointed out that many individuals died during the experiments. *Pulsatilla vulgaris* was the most productive species in the first year, but in the second year, growth was strongly retarded irrespectively of whether plants were cut or kept dry in the previous season.

In the table, ammonia effects on shoot mass are expressed as response ratios (RR) in plants exposed to the highest vs. the lowest concentrations, i.e. treatment nr. 5 vs. treatment nr. 1. Increases in shoot mass due to the ammonia fertilisation (RR>1) are indicated in blue while adverse effects are highlighted in red. The RR assumes a linear response and does not account for the intermediate concentrations, which in the fumigation study in the first year only varied slightly between the set treatments. In the fumigation experiment in 2020, growth reductions were observed in *Dianthus deltoides*, *Carex arenaria*, *Koeleria glauca* and *Antennaria dioica*. Interestingly, RR were also lower than 1 in the second year in the field, indicating that plants which were exposed close to the stables produced less biomass.

Table 29: Growth responses of plants used in the fumigation (2020) and the field gradient study (2021).

Species are sorted after their productivity (sum of two years), with the perennials above and the annuals below. Data are organized after water supply in the dry treatments (DT) and the well-watered (WW) plants as well after plants that were subject to a CUT or those that were only cut once at the end of the season (UNCUT). "RR" is the response ratio and refers to the shoot mass ratio between plants that were exposed to the highest ammonia treatment, i.e. close to the farm (nr. 5) and those that were growing at the most distant location (nr. 1). "CSR" refers to the ecological plant strategies after Grime (2006) and "N" to the nitrogen indicator values after Ellenberg (2001). "x̄" represents mean.

Species	CSR [1]	N [2]	WATER	CUT	RR2020	RR2021	Shoot (g)					Ratio
							2020	x̄	2021	x̄	Total	21/20
Perennials												
Arnica montana	CSR	2	DT	CUT	0,96	2,85	2,76		2,23			
(Jelitto)				UNCUT	1,14		2,57		3,44			
			WW	CUT	0,72	1,12	3,55		3,54			
				UNCUT	1	1,41	3,37	3,06	4,14	3,34	6,4	1,09
Pulsatilla vulgaris		2	DT	CUT	1,29	1,04	3,09		1,58			
				UNCUT	0,97	0,77	3,27		1,72			
			WW	CUT	1,15	0,95	3,56		1,71			
				UNCUT	1,1	1	2,92	3,21	1,81	1,7	4,91	0,53
Carex arenaria	CS	2	DT	CUT	0,7	0,91	1,27		2,92			
				UNCUT	0,55	0,75	1,22		3,22			
			WW	CUT	0,88	1,03	2,14		2,62			
				UNCUT	0,94	0,9	2,07	1,67	3,49	3,06	4,73	1,83
Arnica montana	CSR	2	DT	CUT	1,15		1,82		2,09			
(Weiherberg)				UNCUT	1,19		1,99		2,03			
			WW	CUT	1,12	0,67	2,16		2,32			
				UNCUT	0,84	1,27	2,46	2,11	2,78	2,31	4,42	1,09
Molinia caerulea	CS	1	DT	CUT	1,04	1,56	1,29		2,49			
				UNCUT	0,86	1,37	0,96		2,95			
			WW	CUT	1,02	1,4	1,65		1,81			
				UNCUT	1,08	1,62	1,17	1,27	2,97	2,55	3,82	2,01
Koeleria glauca	CS	1	DT	CUT	0,96	1,03	2,79		0,73			
				UNCUT	0,71	0,74	2,06		0,87			
			WW	CUT	0,95	1,18	3,37		0,74			
				UNCUT	0,88	0,76	2,62	2,71	1,01	0,84	3,55	0,31
Dianthus deltoides	S/CSR	2	DT	CUT	0,71	0,91	1,39		1			
				UNCUT	0,62	0,46	1,48		1,18			
			WW	CUT	0,94	0,86	1,88		1,13			
				UNCUT	0,87	0,77	2,14	1,72	1,02	1,08	2,8	0,63
Antennaria dioica	CSR	2	DT	CUT	0,79	1,35	0,38		0,52			
				UNCUT		0,67			0,63			
			WW	CUT	1	0,89	0,66		0,71			
				UNCUT		0,72		0,52	1,26	0,78	1,3	1,49
Dianthus gratianop.		1				0,93			6,65			
Annuals												
Trifolium arvense	SR	1				0,68			1,43			
Iberis amara		3				1,26			0,43			
Bromus hordeaceus	R/CR	3				1,49			0,37			
Erodium cicutarium	SR					1,42			0,24			
Legousia spec.-ven.		3				0,93			0,08			

We can thus expect that ammonia may have negative effects on the growth of these species. Interestingly, growth reductions were lower in those plants (UNCUT) that had not undergone an intermediate harvest in the previous season.

Our experiments confirm that the effects of ammonia on plants are modulated by drought and mowing. Drought may overall reduce growth of plants and thus the responsivity of plants to ammonia and gaseous air pollutants like ozone. This is due to the fact that the gas exchange and loss of water via transpiration of plants experiencing drought is strongly reduced and that the closure of stomata will prevent the influx of (toxic as well as fertilizing) gases.

Cutting or mowing the plants during the season may on the other hand remove pollutant affected tissues so that re-sprouting of healthy new leaves can often compensate for previously accumulating adverse growth effects. Interestingly, the graminoid species all produced more shoot mass when an intermediate harvest occurred, i.e. the dry biomass (sum) of two harvests was larger as compared to the shoot mass of only one harvest that was performed at the end of the season. Such "overyielding" effects were strongest in *Molinia caerulea* in the first year of the experiment. This is in line with the observation, that in mixtures increased productivity of that species has often been ascribed to resource complementary, the association of mycorrhiza and the release of nutrients (Li et al. 2014). Increased productivity after mowing on the species level will however be due to the removal of growth hormones in the tips of old leaves and the re-sprouting of new leaves in order to quickly maximize the photosynthetic area. However, in the following season tussocks from plants that were subjected to an intermediate harvest formed less biomass than those which were only cut once in the end of the season. Obviously uncut plants were able to keep more nutrients and reserves for the re-sprouting in the next season.

While mean shoot masses of the uncut plants were almost 3 g in both the DT and WW grasses, aboveground biomass was lowered to 2.49 g in the cut DT and even to 1.81 g in the cut WW sub-treatment. Despite the somewhat lower growth in cut plants, *Molinia* plants were still able to sustain a higher biomass production in the second year than in the first year and it was the only perennial species, in which the RR was positive in all four treatments. Obviously, in the field trial the grass was well able to scavenge ammonia from the air and to use the higher nitrogen input close to the stable for increasing its shoot. In the DT, a higher RR was observed in the cut plants while it was the opposite in the plants which had in the previous season grown in the WW treatment. Also, in the study of Leith et al. (2002), the effects of ammonia on *Molinia* were more pronounced and significant in the second year, indicating that effects may be modulated by the internal cycling of nitrogen. However, the realized concentrations of ammonia were much higher in the cited study and plants were not exposed as single plants but were grown in swords (under competition).

Interestingly, the other graminoids, *Koeleria* and *Carex*, responded differently than *Molinia*, but we don't have an explanation for this. In *Koeleria*, the low RR in previously uncut plants may be a result of a denser canopy and that more old leaf sheaths remained in the tussocks so that there was no need to produce new leaves in the second season. As mentioned above, also in other species the removal of resources by an intermediate harvest increased the responsivity to ammonia. We can therefore assume that this comprises a common feature in natural communities exposed to elevated concentrations of ammonia. It may thus be counterproductive in land management to mow seminatural grasslands prone to continuous eutrophication because some species, not necessarily the target species, might be stimulated in growth from nitrogen deposition after the removal of biomass. We might expect e.g. that grasses would boost their growth and outcompete smaller herbs like *Dianthus deltoides* and *Antennaria dioica*. While *Dianthus* in our experiments featured a lowered RR to ammonia in both of the years, mowing and drought in *Antennaria* brought about a positive RR in the field study.

Interestingly, the invasion of grasses at the expense of rare forbs has often been reported in regions exposed to increased nitrogen deposition (Stevens et al. 2006, 2011 citing many studies from the 1980s). Nevertheless, also other factors like the long-term recovery from widespread acidification by sulphur dioxide, altered management practices and climatic change play role in the current vegetation changes in European grasslands (Peppler-Lisbach et al. 2019). It must also be pointed out that the ecology and longevity of plant individuals decide how the populations of different taxa will cope with environmental stresses. While all individuals stemming from a clone of *Molinia caerulea* survived the three years of the experiment, many plants of *Arnica montana* died in the weeks and months after sowing (1st year), during the fumigation study (2nd year) and in the field (3rd year). Obviously, *Arnica* is not a very long-lived species and is generally difficult to maintain over many years, while other robust and long-lived species will stand various stresses and may outcompete the weaker on the long run.

Oligotrophic montane grasslands have seen marked species changes in recent times and severe drought has been shown important for “species filtering” (Stanik et al. 2021). Also, in the performance and survival of arnica, drought seems to play a significant role. In the study of Stanik et al. (2020), fitness related functional traits of *Arnica montana* showed a greater variability with increasing aridity and the authors found that under montane conditions variation was lowest. Our results confirmed that arnica and most other species responded strongly to drought. Growth reductions were a common response, but in contrast to arnica, most individuals were able to survive the treatments and treatment combinations (drought x mowing x ammonia). In the well-watered *Arnica montana*, 64 % (Jelitto) respectively 44 % (Weiherberg) of the plants survived until the end of the experiment. In the DT treatments the survival rate further went down to 26 respectively 16%, whereas in the other six perennial species it was over 94%. While the death rate in Arnica was generally high and even higher under dry conditions, the effects of drought on shoot growth were not different from other species, both in the year when the drought was applied and in the following year.

Since in the field gradient study, i.e. in the second year of the experiment and the third season of the plants´ existence, most of the selected individuals flowered, we are sure that we covered a full life cycle of the tested plant species. The transition of the plants into the reproductive stage did not occur in the closed chambers, most probably due to the high temperatures in the greenhouse. In experiments under controlled conditions, it is almost impossible to realize natural growth conditions. Neither the edaphic nor the climatic conditions will be comparable to the environmental situation. In our fumigation study it was also not possible to achieve the set ammonia concentrations, i.e. a concentration range from 0 to 10 µg m^{-3}. Nevertheless, concentrations were low enough to not create acute symptoms. In the second year, in the field gradient study we achieved a concentration gradient very close to the envisaged levels and also the climate represented a realistic situation. We therefore conclude that the results gained in the second season were reliable with regard to responses of single plant species not growing in competition with others. In order to get an ecologically meaningful idea on what low levels of ammonia will do in plant communities, one would have to perform experiments in established natural or semi-natural vegetation.

4 Suggestions for future research (WP3)

4.1 Field fumigation experiments

4.1.1 Possible technologies

Based on the evaluation of the peer-reviewed literature, we call for an accurate documentation of the study conditions and methods that are being used in experiments on the effects of ammonia on plants. In order to update or justify the suggested low critical levels for the gas, more research is necessary to derive solid dose-response relationships between measured ammonia concentrations and relevant biological endpoints. In order to not just extrapolate from results achieved at higher concentrations, we suggest that research on ammonia should focus on the chronic effects potentially occurring at ambient levels <10 µg m^{-3}. Hard response parameters will be the growth responses and changes in nitrogen concentrations on the plant level, whereas changes in competitive abilities and biodiversity will relate to cumulative long-term effects of the nitrogen deposition (Pitcairn et al. 1998). We also suggest to underpin research on the presence and absence of N-sensitive lichens with basic research on the involved ecophysiological and metabolic effects of ammonia on the symbiosis between algae, fungi and bacteria.

Indoor and outdoor fumigation experiments should at least last for a complete season in order to maximise the potential effects of ammonia on the growth of vascular plants. In general, plants may be more responsive in the earlier growth stages, but a complete life cycle should be addressed to identify potential interactions of ammonia with the phenology and senescence of plants. Since ammonia will interfere with the plant nutrition, we may expect changes in the allocation patterns and source-to-sink relationships. When aiming at the study of lichen and bryophyte plant species, it will be necessary to select uniform material from pristine areas and one would have to include twigs of a pre-defined diameter of the host tree. Vascular plant species should be grown from seeds and the selected species should be representatives from the group of rare nitrogen-sensitive species, e.g. moorlands or acidic grasslands. Substrates of the potted plants should be representative of the natural conditions where the plants grow and should be supplied with a low amount of ^{15}N labelled nitrogen fertilizers. Isotopic analyses can help to clarify how much of the airborne nitrogen was metabolized and in which plant parts the nutrient will be concentrated. Since plants will experience mostly unnatural climatic and light conditions in chamber-based controlled fumigation experiments, it may be favorable to apply active approaches with pre-grown vascular plants in ammonia gradients in the field. Plant responses can be related to the measured ammonia concentrations, but growth conditions must not vary along the gradient. Specimens are therefore exposed under standard conditions using the same growth medium, water and nutrient supply and terms of exposure (e.g. height).

As mentioned in chapter 2.2, fumigation of plants with ammonia in the early years had been based on the use of pure (technical) NH_3 supplied from gas flasks to the air inlet of small closed growth chambers Very high concentrations were used to study whether growth of crop plants could be stimulated by nitrogen containing gases, but low concentrations and wild plants only later were focused at.

Closed and open chambers, other plant species than crops and lower concentrations over longer exposure intervals were first used by Dutch groups and it became clear that due to bi-directional fluxes and low compensation points also crops and semi-natural vegetation may release ammonia at times[4]. In Germany, adverse effects of ammonia were addressed at the Federal Research Institute of Agriculture (FAL, Adaros and Dämmgen 1994) and at the University of Gießen (Fangmeier et al. 1994). Unfortunately, the fumigation and the recording of ammonia levels were not satisfactory in latter study, but at the mentioned German agricultural research institutions there might still be solid know-how for future air pollution impact studies It might also be possible to incorporate ammonia fumigation into existing global change research facilities, namely the FACE systems operated at Braunschweig and Giessen.

In the late 1990s, it became clear that the chronic responses in unfertilized wild plant species and the nutrient status of ecosystems needed to be looked at differently than the acute or toxic responses one might expect due to accidental releases of ammonia e.g. from industrial refrigerators and fertilizer production facilities. Best approaches would be to develop field-based fumigation systems to study the effects of ammonia on established natural vegetation.

WP3 of the presented project consisted of a feasibility study for a field-based ammonia experimental facility in Germany. The only place currently where such an exposure facility has been set up is the Scottish Whim Bog in the Southwest of Edinburgh. Experiments were started in the year 2002 and provide a more or less well quantified ammonia (NH_3) concentration and deposition gradient to an ombrotrophic bog. The system is best described in the paper by Leith et al. (2004). The Centre for Ecology and Hydrology (CEH), who set up the study site, has recently built other similar ammonia exposure systems in a Scottish forest and in Sri Lanka (personal communication Matt Jones, reports on the most recent study sites were not available, yet).

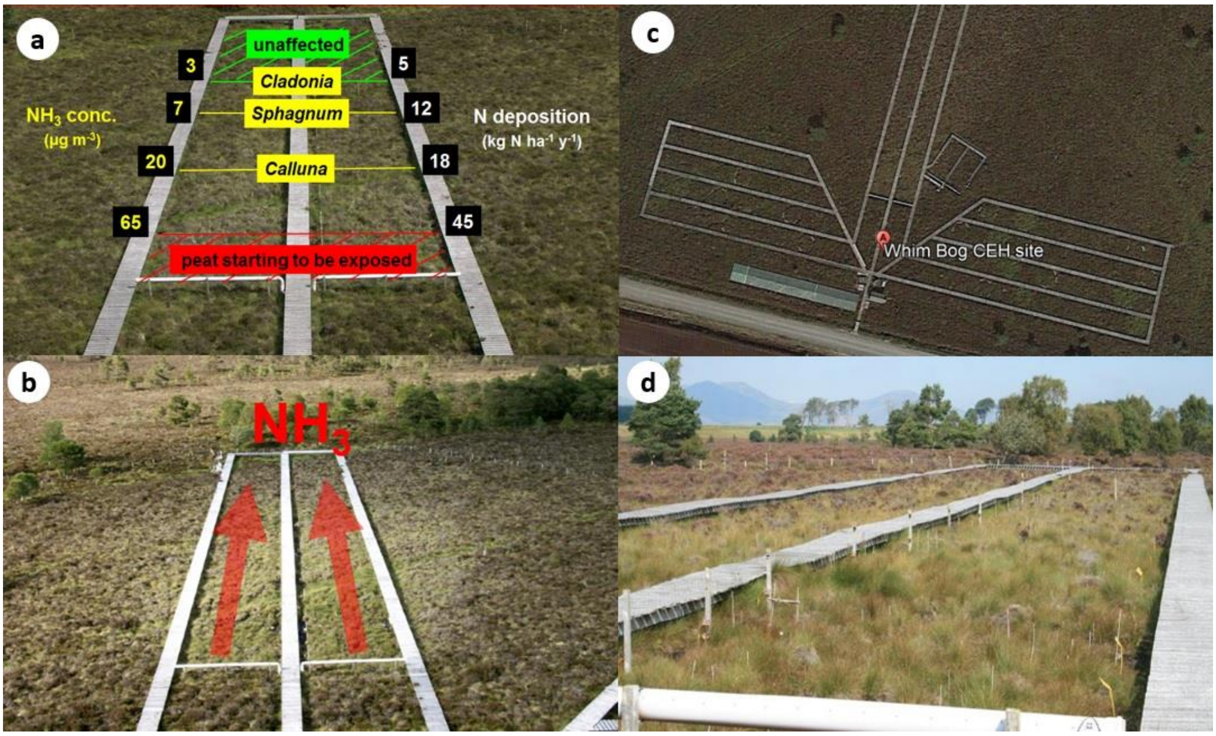
Based on the information collated in chapter 2.2 and on our own experience with the field gradient study (chapter 3.3), here we come up with ideas where and how to perform ammonia related studies in the future. The mentioned Whim Bog and the CEH operated sites are the only field fumigation systems, in which ammonia is released directly into an established vegetation. Information on the study site and discussion of the Scottish ammonia fumigation facility are summarized in the text box below and are used to suggest the development of other methodologies. More often, open top chambers (OTCs) have been used to manipulate the air quality in canopies of crops and stands of natural vegetation. Most of such studies investigated air pollutants like sulphur dioxide and ozone and only a few made use of ammonia. We suggest that the use of OTCs creates strong microclimatic artefacts so that chamber less systems should in any case be the better alternative.

Since tuning in and controlling the concentrations of pure (or diluted) ammonia at low concentrations of less than 10 µg m^{-3} is technically extremely challenging, vaporisation of the gas from diluted ammonia solutions was used in the studies of Leith et al. (2002) and Jones et al. (2013). In latter study, high concentrations were present in the field chambers after the weekly re-charging of the solutions, while in the study of Leith et al. (2002) there was a steady volatilisation of the gas from 1% solutions of NH_4OH. Unfortunately, detailed information was lacking in both articles on how the systems were set up and data of the four weeks measuring intervals (passive samplers) of realised concentrations as well as the climatic conditions inside the chambers were not given as well. It will be necessary in future fumigation experiments to have a clear picture of the ammonia concentrations in the experiments, both of the gradients in the field and the spatial distribution as well as the temporal fluctuations. There should at least

[4] The ammonia compensation refers to the equilibrium between the ammonia concentrations in the air and in the sub-stomatal cavity of plants. It is determined by the temperature of the leaf, the microclimate and the ammonium (NH_4^+) concentration (nutrient status) and pH of the apoplastic solution. During the lifetime of plants, even the unfertilized vegetation, bi-directional fluxes of ammonia will occur. While young N-demanding plants are a sink of ammonia, the opposite can be true in old senescing and stressed plants. It is thus important to study plants in their natural setting, over complete life cycles and multiple seasons.

be one device by which concentrations will be measured online in the real time mode and to which passive samplers can be calibrated. For the spatial distribution of ammonia in the field, we suggest using passive samplers at a weekly resolution.

The Whim Bog long-term ammonia experiment



Source: Information was collated from various sources: personal communication with Neil Cape and Mathew Jones, papers from Leith et al. (2004), Sheppard et al. (2011), van Dijk (2017) and Levy et al. (2019), and previous versions of the following websites http://www.whimbog.ceh.ac.uk and https://www.ecologicalcontinuitytrust.org/whim

Photos show the set-up of the fumigation system and the development of the vegetation over time: a) indicates the ammonia and deposition gradient in the lee of the gas outlet (white tube), where ammonia is released. Note that the numbers are mean concentrations and nitrogen deposition rates that were corrected for the canopy resistance of the vegetation. According to the operators of the facility, ammonia concentrations reach occasional peaks of over 1600 µg m^{-3}. b) shows the “bleaching effects” due to ammonia observed after a few years of fumigation. While *Cladonia* lichens, *Sphagnum* mosses and the dwarf shrub *Calluna* heath were the first to die, other vascular species like *Vaccinium myrtillus, Erica tetralix and Eriophorum vaginatum* survived and even increased in cover. c) shows a top view of the site and d) proves the strong dominance of grass like species.

Strengths and weaknesses of the study are:

- Constant and semi-controlled release of ammonia, but occasionally very high peak levels near the outlet and the high temporal and spatial variability of the levels cannot be measured.
- The system is operated in a natural ecosystem, but the vegetation is not homogenous; peat extraction nearby and former ditches in the field may be problematic, but human influences exist in all European ecosystems, so that the choice of uniform sites will always be difficult.

- Since only one gradient has been established in a static system, the study has no true replicates; it also creates (cumulative) carry over effects over time. This would, however, be the case in all long-term field studies using static systems.
- NH_3 addition takes place only when wind comes from the south to create a uniform gradient. However, no feedback control system was available in the first years that can be based on actual levels. Recently, ring Cavity Ring-Down sensors (CRDS) were installed, which in the future may be used to also feed-back the fumigation system.

Based on the studies by Leith et al. (2002) and Jones et al. (2013) and the experiences from our own experiments in WP2, in which a gentle vaporisation of liquid ammonia was used in greenhouse compartments, we suggest an approach that could be realized in the field. The idea is to evaporate NH_4OH solutions from linear structures which can be freely arranged in a natural vegetation at different densities, so that an artificial field gradient can be created. Ammonia levels may be adjusted via different concentrations of liquid ammonia and different flow rates. Such a flexible system could be set up for up to three years in different locations and vegetations and would not create the above-mentioned carry-over effects, i.e. a strong eutrophication at the blower outlets, where pure ammonia is released.

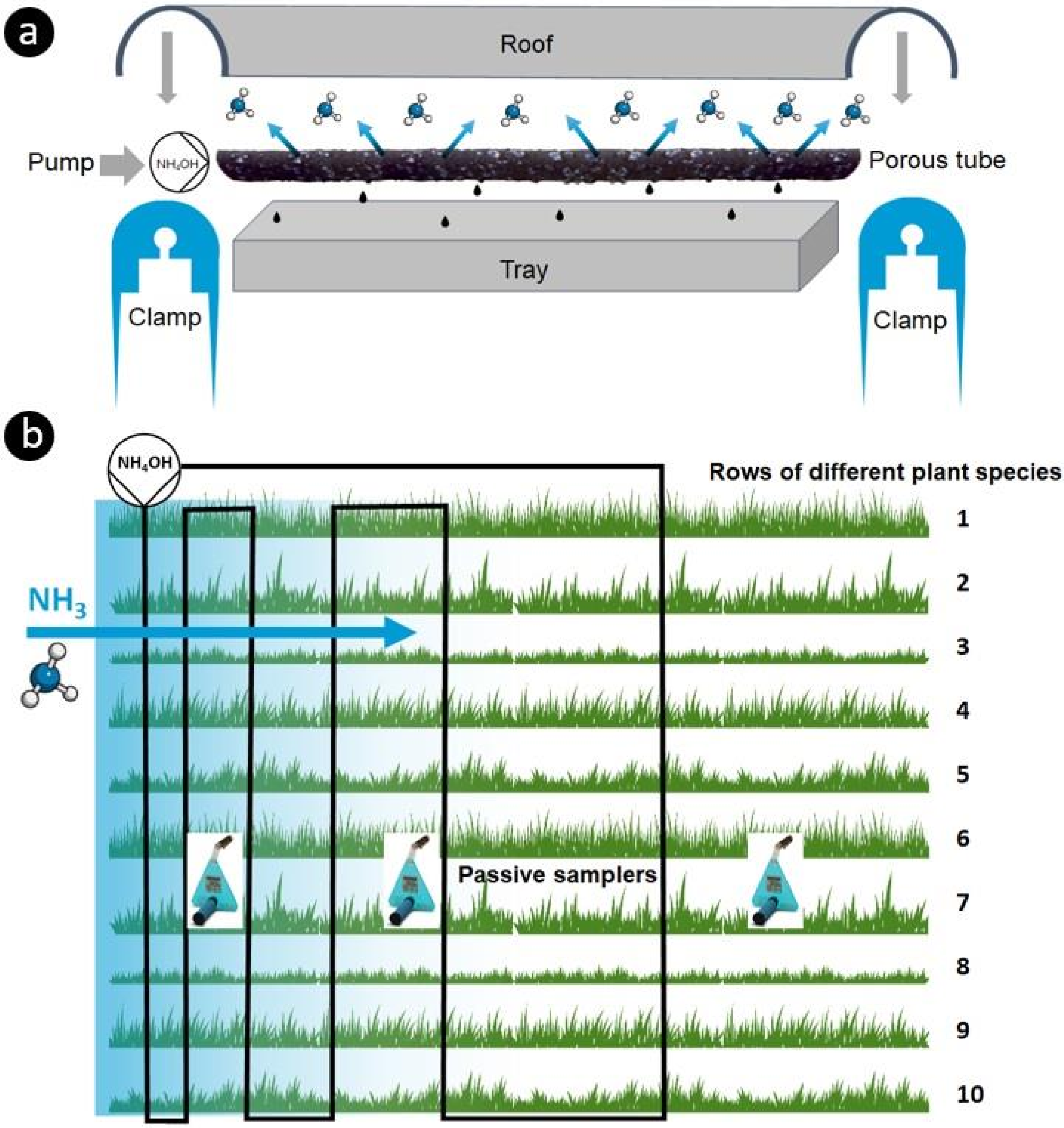
Evaporation of NH_3 could derive from the surface of microporous irrigation pipes and the liquid ammonia would be led via pumps at low pressure through these pipes in order to allow the ammonia to be completely transferred into the gas phase. Nevertheless, a collector tray needs to be installed underneath the pipe in order to take up liquid ammonia, which otherwise would drip into the vegetation and fertilise the soil. At the same time, a rain shed needs to be installed above the porous pipe in order to prevent the washing out of ammonium and the drift of liquid ammonia drops from the line. The tube would therefore have to be placed in e.g. a half pipe and a broader half pipe would have to protect the porous hose from above. Figure 35 gives a sketch of the suggested vaporisation-based approach. The upper part (a) shows a close-up of the system and the lower part of the graph (b) gives an idea of how the tubes could be arranged in a vegetation to create ammonia gradients. The aim would be to create a concentration gradient from 3 to 30 µg m^{-3} to be able to assess sub-chronic responses of plants under realistic conditions in the field.

In order to construct a flexible and technically reliable system for the controlled evaporation of liquid ammonia, an EU wide tender would have to be issued for the development and exhaustive tests of a robust and simple prototype. Potential applicants will be private engineering offices that deal with control and regulation technology as well as university and federal institutes for e.g. agricultural engineering.

Provided a substantial funding of the equipment, running costs and technical and scientific staff, it will be possible to set up ammonia exposure facilities in Germany and elsewhere. We suggest to set up systems at four locations using a system that relies on the gentle evaporation of ammonia from perforated pipes. To guarantee openness to technological ideas, flexible systems using a concentration-controlled release of pure ammonia can be still an alternative to the gentle evaporation from NH_4OH fluids, provided that they do not create microclimatic artefacts. A competitive tender could clarify which of the approaches would be more flexible, practical and economical.

Figure 35: Scheme of a tube-based ammonia exposure system

a) shows a section of the suggested system composed of a microporous tube for the vaporisation of ammonia and a rain shed above and tray below the tube to prevent washout of ammonium into the vegetation. Clamps can be stuck in the ground every 2 m to fix the linear structures of the system. b) shows an example how the tube could be placed in a vegetation or a test field to create an ammonia gradient from left to right.



Source: Own illustration, University of Hohenheim.

4.1.2 Possible sites

It would be desirable to have two sites in a mire or a fen, one in the North and another in the South of Germany. Furthermore, two more facilities in an acidic and a calcareous grassland should be set up, one in the West and another in the East. Due to their large volumes, height and standing biomass, forests will have to be excluded from ammonia fumigation experiments, since that would afford high investments in equipment and running costs. However, forest fumigation systems using the release of gases from Teflon tubes have been developed for ozone and carbon dioxide and similar approaches could as well be used for ammonia. In order to focus on ammonia effects on trees in the establishment phase, one could however install flexible systems in forest sites, where due to strong drought and calamities clear-cuttings had to be made recently. It would be interesting to follow the effects of ammonia and eutrophication in the pioneer phase of forest growth, species composition, wood growth and resilience to climatic change.

Bobbink et al. (2022) presented a revision of the empirical critical loads for nitrogen and concentrated on research that was published after the year 2011. The editors and authors of the report concluded that more research and data will be required to establish critical loads for several grasslands and hay meadows, Mediterranean vegetation types, wet swamp forests, mires and fens and several coastal habitats. Many of the EUNIS habitat types have not been investigated and long-term nitrogen addition experiments are necessary to address the effects of the deposited NOx or NHy in order to be able to determine the critical loads for the two forms of nitrogen separately in the future. More studies in both low and high N regions should be set up and it will be important to in the future also address the speed and extent of recovery from long lasting nitrogen deposition also in the light of the ongoing climatic change.

Since critical loads and critical levels for nitrogen compounds are interrelated and ammonia contributes much to the adverse effects of nitrogen deposition, a general recommendation is to set up fumigation experiments at those sites where nitrogen addition experiments had already been established preferably in those habitat types where empirical critical loads have been studied recently. Ideally, future experiments should be incorporated in long-term ecological research initiatives or platforms on global change to bundle resources and research infrastructures and to develop new benchmarks for nitrogen research in Germany.

In the next paragraphs, we will therefore collate information on several research platforms, where nitrogen related research had been performed in Germany during the last decades and will mention ongoing research initiatives where ammonia research could best be integrated in the future. While eutrophication related research was widespread in the 1980s and 1990s, the majority of nitrogen addition experiments was not continued after the year 2000. Meanwhile, most researchers have retired and most of the underlying information on how the experiments were originally performed is unavailable. Furthermore, it has been shown that the long-term effects of nitrogen deposition on the biodiversity of forest (understorey) species and vegetation change may only be perceivable in certain but not in all forests (Roth et al. 2022). Nutrient poor ecosystems with short vegetation will respond more strongly and rapidly to nitrogen deposition, but studies in such communities are overall very scarce in Germany.

One of the first EU projects, in which nitrogen saturation or N addition experiments were performed, was the NITREX initiative, which lasted from 1991 to 1993 and focused on forest sites in various European countries (European Commission 1993). In Germany, it was performed by the Forestry Unit of the University of Göttingen in the Solling mountains and focused on the interactions between acid rain and nutrient deposition. It might be worthwhile to re-visit some of the sites, especially those where the forest floor vegetation was recorded. It is unclear whether long-term data are available and until when the nitrogen addition lasted. The research project did not consider gaseous N-components and at those times, no efforts were made to monitor ammonia.

In contrast to the N addition experiments in forest ecosystems in Europe, many broad-scale studies were set up in the 1990s in semi-natural grasslands in other regions, e.g. in the US Prairies and the steppes of Inner Mongolia. The database PlantNE[5] lists 398 sites, where N addition took place according references published between 1982 and 2018. Only in one of the five sites, which were operated in Germany, the vegetation type was mentioned. It was a dry

[5] https://zenodo.org/record/3359810

heathland in the Lüneburger Heide and the chosen study plots were fertilised in 2006 and 2008 with NH_4NO_3 (Falk et al. 2010).

Only few German partners were involved in the NitroEurope project (2006-2011)[6], and research focused on N fluxes and budgets rather than on ecosystem effects. As mentioned several times before, research on the effects of ammonia in natural vegetation almost completely stems from Scotland and focuses on the widespread Moorland vegetation. In Nordic countries, it may be very easy to get access to such vegetation and to start long-term manipulation experiments. Because of the high protection status of bogs, fens, heath and steppe-like grasslands in Germany, it will have to be clarified with the authorities and site managers, whether nitrogen addition and ammonia fumigation studies can be performed in such, most likely protected Natura2000 sites. However, investigations on nitrogen impacts without the manipulation of the vegetation have been done in the prominent Mainz sand dunes and steppe relics (Franzaring et al. 2010). Semi-natural grasslands had also been the focus of long-term fertilization experiments in the south west of Germany, only some of them Natura2000 sites. 14 species-rich grasslands from different habitat types had been investigated for over 35 years including species relevés and productivity measurements (LUBW 2009). Although the sites had been studied extensively for over 20 years, it would take large efforts to start new research there since the sites had never been integrated into existing research infrastructures.

Although the main focus was not on nitrogen deposition, many long-term experiments with an agricultural background have been established in Germany and are still running that deal with the fertilisation of semi-natural and managed grasslands. Some of these sites, especially the oligotrophic systems, might also be interesting for field studies using ammonia fumigation. In the framework of the BonaRes[7] initiative of the German Federal Ministry of Education and Research, 33 grassland sites have been identified by Grosse et al. (2020) and Dönmez et al. (2022), where fertilization experiments are taking place for more than 20 years. We suppose that some of the sites will be connected to and maintained by research institutes and universities and therefore suggest to include them in a general search list for suited places in Germany, where ammonia fumigation experiments could be performed.

Since a multitude of often uncoordinated short-term ecological field experiments had been performed in Germany from the 1980s to the 2000s at many different universities and research centres, DFG, Fraunhofer and Helmholtz centres felt the need to better structure the ecological research and to make suggestions for so-called "observatories" focussing on and bundling long-term environmental research in the future (UFZ 2018)[8]. The goal of LTER-D is to create unique long-term datasets, which cover different species groups and environmental variables.

However, none of the existing projects including the DFG Biodiversity exploratories[9] is currently addressing nitrogen deposition and the effects of gaseous air pollutants like ammonia. We therefore recommend to eventually set up new infrastructures, in which free air fumigation systems can be installed at sites with short semi-natural vegetation, like grasslands, heaths, mires and bogs. Forests should be excluded in the initial stage because of the technical challenges in tall and voluminous canopies. As has mentioned above, it would still be an option to include ammonia fumigation plots in a forest research platform in the near future. It will be interesting to address effects of N addition in young pioneer forest vegetation and the interactions with climatic change. Based on the existing know-how, e.g. at the Thünen Institute

[6] http://www.nitroeurope.ceh.ac.uk/about

[7] BonaRes - Boden als nachhaltige Ressource für die Bioökonomie - Soil as a sustainable resource for the bioeconomy

[8] Information on the German LTER-D network can be found under https://www.ufz.de/lter-d/index.php?en=42518

[9] https://www.biodiversity-exploratories.de/de/

in Braunschweig, whose researchers for over 25 years were and are operating a FACE system in an agricultural setting, it would be an idea to develop small fumigation systems like those mentioned above, which can easily be installed in various natural ecosystems including pioneer forests and ecological observatories e.g. those listed in LTER-D. One could also think of combinations between different air pollutants (ozone, NOx and ammonia) and carbon dioxide and could also include N additions in subplots.

Another relevant network to be mentioned is the Nutrient network[10]. The initiative addresses managed and unmanaged grasslands and five sites are being operated in Germany by different universities and sites, namely at Papenburg, Bayreuth, Jena, Freiburg and Bad-Lauchstädt.

Since peatlands are the most susceptible habitat type to nitrogen additions and the protection of such ecosystems is one of the most important options of the climate related national strategy (*Nationale Moorschutzstrategie*), we recommend setting up long-term research in representative regions, e.g. in the lowlands of Northern and Northeastern Germany and in the pre-alpine moorlands. While some of the LTER-D observatory sites might be well suited, we also suggest that research could maybe also be initiated in the four pilot project regions, which have recently been selected to protect peatlands by the ZUG (Zukunft – Umwelt – Gesellschaft) project manager[11]. Other ongoing projects for peatland protection and paludiculture where ammonia research could be initiated are listed by the Federal Agency for Nature Conservation (BfN)[12] and the Greifswald Moor Centrum[13]. Ideally, such sites should already be equipped with technical devices for the determination of climatic parameters and greenhouse gas fluxes since the release of CO_2, CH_4 and N_2O from wetland ecosystems will greatly be affected by the deposition of nitrogen species.

As has been mentioned before, in the early 2000s, several projects were run in Germany and the EU that exclusively dealt with the measuring of the fluxes of methane, NOx and carbon dioxide but did not address their ecological impacts. Standard protocols and a guideline have recently been established by Fiedler et al. (2022) and could be used to also determine the fluxes of ammonia in future experiments on the biological effects of ammonia. Former research sites operated by the Thünen Institute for Climate-Smart Agriculture e.g. in Northern Germany[14], [15] and the still existing infrastructures should thus also be considered for the establishment of research sites, in which ammonia fumigation systems can be realized or incorporated.

We recommend that funding should be made available in the next years via national and European sources to prepare and tender a call for ammonia related research in oligotrophic environments in the near future. We suggest that dry and moist and calcareous and acidic ecosystems should be addressed since the effects of ammonia will be modified by the ecology of the receiving ecosystem. At least four sites should thus be chosen in Germany and research should at least be done over five years to address mid to long term effects. As has been mentioned above, we recommend having one site each for a peatland and a fen in Northern and Southern Germany and a dry acidic and a calcareous grassland site in Western and Eastern Germany. While some ideas for research in peatlands were described above, the selection of dry

[10] https://nutnet.org/field_sites, NUTNET is hosted by the Department of Ecology, Evolution, and Behavior at the University of Minnesota

[11] https://www.z-u-g.org/aufgaben/pilotvorhaben-moorbodenschutz/

[12] https://www.bfn.de/handlungsbedarf-zum-moorschutz#anchor-3809

[13] https://greifswaldmoor.de/projects.html

[14] https://www.thuenen.de/de/fachinstitute/agrarklimaschutz/projekte/nitrosphere

[15] https://www.swamps-projekt.de/

grassland study sites would have to be started from the scratch. Some of the technical challenges involved in such projects are outlined in chapter 4.1.1.

4.2 Gradient studies

Chapter 2.2.3 gives an overview of the published gradient studies that focused on the study of potentially adverse effects of ammonia in the lee of livestock facilities and in WP2 (Chapter 3.3), we adopted an approach, in which we actively exposed pre-cultivated plants along an ammonia field gradient.

To limit potentially adverse effects on nature, large stables with many animals and presumably high ammonia emissions are licensed only at locations away from sensitive targets, i.e. nature reserves like heath- and moorlands. Since N-sensitive natural vegetation is not present there, it will thus not be possible in such places to address effects of ammonia on plants most prone to eutrophication. One could, however, address the wild plant species, often nitrophytes, available in the field and perform visual assessments and analyses of e.g. the tree foliage or lichens and mosses collected at sites at different distances from the source. The objectives could be to relate the presence of certain species or foliar nitrogen contents to the ammonia plumes released from livestock operations. Such passive approaches consider the vascular plants and lichen and bryophyte species growing in the field and address the established vegetation in the natural, but often too variable setting. They are prone to many artefacts that will inevitably be created by varying soil conditions and a change in species composition along the gradients, which will eventually override the ammonia effects.

However, neither active nor passive approaches in field gradient studies will be able to separate the effects of reduced (NH_x) and oxidised (NO_y) nitrogen species that are deposited as gases, wet or dry particulate components. Often the responses are more pronounced for the deposition of oxidised than for reduced nitrogen species (e.g. Roth et al. 2021), but this greatly depends on whether the vegetation grows on acidic or calcareous substrates. It must also be pointed out that responses like increased growth, foliar N-contents, changed isotopic patterns etc. that are determined in a target species are not negative effects per se. Only when looking at multi-species responses, e.g. changes in competition one will be able to get a clearer picture of the ammonia driven ecological effects.

In contrast to passive approaches, active biomonitoring is a highly standardised approach, excluding the mentioned artefacts in the responses of plants growing in their natural habitat. Such approaches are being used for a defined project duration, so one will be able to also address the time and dose it takes until ammonia effects are sensed by plants.

A classical biomonitoring method that has been used for decades in numerous regional air pollution impact studies in Germany is the standardised grass culture method (VDI 2020, Guideline 3957 Part 2), in which pre-grown *Lolium multiflorum* are exposed at a standard height in self-watering exposure systems. The equipment and the approach are very well suited for the exposure of all types of plant species to study the effects of atmospheric ammonia. Standardised grass cultures had been designed as accumulation indicators, e.g. to prove how much heavy metals or sulphur are taken up from the polluted atmosphere. The approach is still being used in routine monitoring networks in the States of Northrhine-Westfalia (Wirkungsdauermess-programm) and Bavaria and the results can be related to the existing EU threshold levels for certain pollutants in fodder.

We therefore suggest to apply a similar method as has been used in WP2. Pre-grown N-sensitive lower and vascular plant species can be exposed actively as bioindicators along the ammonia gradient in the lee of large stables. When designing a field gradient study, the following general approach should be followed:

- At first, identify a large ammonia emission source, e.g. a large livestock facility and make contacts to the owner and respectively, the farmers owning the land where you will set up the monitoring stations
- Identify the type and number of animals present in the stables and use typical emission factors to calculate annual ammonia emissions. Also include information on the outlet height of the emissions and the stable type
- Use a dispersion model and a digital elevation model and the typical regional or local wind direction patterns to determine, into which direction most of the emissions will be blown
- Identify the emission gradient and based on the dispersion model, determine where to roughly locate the stations along the gradient. Later in the field, check the accessibility of the stations
- Determine what kind of vegetation is present in the field. Map the trees, vegetation types and which crops are cultivated on the fields
- In the field, determine exactly (note the coordinates with GPS) where you will place the monitoring/exposition stations; make sure that there are no trees and obstacles at the stations and try to avoid public roads to minimize effects of vandalism
- Set up a research/monitoring plan for the scheduling of actions, the personnel involved and how and when the material is going to be transported to the sites
- Purchase all the materials necessary, i.e. nutrient-poor cultivation substrate, seed materials, pots, glass fibre wicks, pots and watering system, exposure poles (see lists in VDI 2020, Guideline 3957 Part 2)
- Pre-cultivate enough plant material in a greenhouse, make sure that the plants grow well and have no pests
- Take samples from the substrate and analyse nitrate, ammonium, total nitrogen concentrations and ^{15}N as a reference for the analyses at the end of the season
- Monitor ammonia concentrations (and if possible, NOx) at the chosen stations a few weeks before the biomonitoring plants are brought to the field to ensure that the gradient compares well to the output of the dispersion model
- Expose four plants (replicates) and the watering systems at each of the stations. Pot surfaces should be at 1.5 m
- For watering of the plants, i.e. the supply of water to the self-watering system, use deionised water to not add nutrients to the biomonitoring system
- During the research, monitor ammonia and climatic conditions (temperature, relative humidity, *bulk* precipitation wind speed and direction) at each of the stations
- Collect precipitation samples each time you change the passive samplers (bi-weekly or weekly). Keep these samples cool in a laboratory fridge until you monitor nitrate and ammonium to calculate the *bulk*-N deposition (NHx and NHy)

- During the field campaign, apply non-destructive assessments to describe the vitality and growth of the labelled plants (4 replicates per station). These should include plant height, phenology (flowering, number of side shoots, senescent leaves etc.) and leaf greenness (using a SPAD meter)
- In the end of the vegetation period, move all the plants and the materials to the lab. Determine aboveground and belowground dry biomass of each of the plants. Take samples of the biomass for analyses of element concentrations (N, P, K) and ^{15}N.

Figure 36 shows an example of a biomonitoring system using the grass *Molinia caerulea*. The grass species is common in dry and wet acidic grasslands and has often been shown to be increasing its presence and cover in areas subjected to strong eutrophication. Indeed, in our field study Purple Moorgrass showed higher growth rates in relation to higher ammonia concentrations near the stables confirming that even after shorter periods, the species can act as a bioindicator. Instead of sowing the species, we used equally sized ramets, i.e. clonal material, which were planted in a nutrient poor substrate. None of the plants died during the two years trials confirming the robust nature and longevity of the plant species.

Figure 36: *Molinia*-Biomonitoring system

The photos show the preparation of plant ramets (left) and the self-watering exposure system in the field (centre). Using the equipment, pots of pre-cultivated plants can be sustained with additional watering for several weeks. On the right, roots of a Molinia plant and the interwoven glass fibre wicks are shown after taking out the pot from the exposure system.

Source: Own illustration, University of Hohenheim.

As has been mentioned above, changes in competitive abilities in mixed systems will probably show stronger effects of the nitrogen inputs than just using the biomass increment of a single species. Apart from the competitor *Molinia caerulea*, we suggest using a herb as a second, less competitive species. One option could be the slow-growing clover *Trifolium arvense*, which in our field gradient study showed a retarded growth at higher ammonia concentrations. In order to develop a biomonitoring system that is based on changes in competition, we recommend a pilot study using clones of the Purple Moor Grass as the competitive species and various smaller herbs as the outcompeted species. The approach should be developed in the lee of a large farm to capture the full gradient from highly elevated to background ammonia concentrations.

If the method should prove feasible, a guideline should be developed for the standardised implementation of the test system in future studies. We recommend to use the method in e.g. national or regional citizen science projects or in international monitoring programs e.g. the ICP Vegetation. However, basic research needs to be funded for at least 3 years prior to the inclusion in routine monitoring programs.

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Annex 2

List of abbreviations and description of plant parameters (alphabetical order) presented in the tables, annexes and text of the report and as headers in the original data files.

Abbreviation	Determined in year	Description
ARNC.12	2020, Fumigation Study	Arnica, C% 1st and 2nd Senescent fraction
ARNC.34	2020, Fumigation Study	Arnica, C% 3rd and 4th Senescent fraction
ARNC.5	2020, Fumigation Study	Arnica, C%, 5th senescent faction
ARNC.5.1	2020, Fumigation Study	Arnica, C%, 5th senescent faction
ARNC.6	2021, Field Gradient Study	Arnica, C% of 6th senescent fraction
ARNC.7	2021, Field Gradient Study	Arnica, C%, 7th senescent fraction
ARNC.8	2021, Field Gradient Study	Arnica, C%, 8th senescent fraction
ARNC.GREEN21	2021, Field Gradient Study	Arnica, C%, green biomass 2021
ARNC1312	2020, Fumigation Study	Arnica, 13C 1st and 2nd Senescent fraction
ARNC1334	2020, Fumigation Study	Arnica, 13C 3rd and 4th Senescent fraction
ARNC135	2020, Fumigation Study	Arnica, 13C, 5th senescent faction
ARNC136	2021, Field Gradient Study	Arnica, 13C, 6th senescent fraction
ARNC137	2021, Field Gradient Study	Arnica, 13C, 7th senescent fraction
ARNC138	2021, Field Gradient Study	Arnica, 13C, 8th senescent fraction
ARNC13GREEN21	2021, Field Gradient Study	Arnica, 13C, green biomass 2021
ARNCN12	2020, Fumigation Study	Arnica, CN of 1st and 2nd fraction of senescent leaves
ARNCN34	2020, Fumigation Study	Arnica, CN of 3rd and 4th fraction of senescent leaves
ARNCN5	2020, Fumigation Study	Arnica, CN of 5fth fraction of senescent leaves
ARNCN6	2021, Field Gradient Study	Arnica, CN, 6th senescent fraction
ARNCN7	2021, Field Gradient Study	Arnica, CN, 7th senescent fraction
ARNCN8	2021, Field Gradient Study	Arnica, CN, 8th senescent fraction
ARNCNGREEN	2021, Field Gradient Study	Arnica, CN, green biomass 2021
ARNGREEN2021	2021, Field Gradient Study	Arnica, green biomass 2021
ARNN.12	2020, Fumigation Study	Arnica, senescent leaf N%, 1st and 2nd combined
ARNN.34	2020, Fumigation Study	Arnica, senescent leaf N%, 3rd and fourth combined
ARNN.5	2020, Fumigation Study	Arnica, nitrogen concentration (%) of 5fth fraction of senescent leaves
ARNN.6	2021, Field Gradient Study	Arnica, N%, 6th senescent fraction
ARNN.7	2021, Field Gradient Study	Arnica, N%, 7th senescent fraction
ARNN.8	2021, Field Gradient Study	Arnica, N%, 8th senescent fraction
ARNN.GREEN21	2021, Field Gradient Study	Arnica, green biomass 2021
ARNN1512	2020, Fumigation Study	Arnica, senescent leaf 15N, 1st and 2nd combined
ARNN1534	2020, Fumigation Study	Arnica, senescent leaf 15N, 3rd and fourth combined
ARNN155	2020, Fumigation Study	Arnica, 15N of 5fth fraction of senescent leaves
ARNN155.1	2020, Fumigation Study	Arnica, 15N, 5th senescent faction
ARNN156	2021, Field Gradient Study	Arnica, 15N of 6fth fraction of senescent leaves
ARNN157	2021, Field Gradient Study	Arnica, 15N, 7th senescent fraction
ARNN158	2021, Field Gradient Study	Arnica, 15N, 8th senescent fraction
ARNN15GREEN21	2021, Field Gradient Study	Arnica, 15N, green biomass 2021
ARNNTOT	2020, Fumigation Study	Arnica, total N in mg
ARNNTOT12	2020, Fumigation Study	Arnica, total N 1st and 2nd Senescent fraction
ARNNTOT2020	2020, Fumigation Study	Arnica, total N in 2020
ARNNTOT34	2020, Fumigation Study	Arnica, total N 3rd and 4th Senescent fraction
ARNNTOT5	2020, Fumigation Study	Arnica, total N, 5th senescent faction
ARNSEN12	2020, Fumigation Study	Arnica, senescent leaf mass, 1st and 2nd combined
ARNSEN34	2020, Fumigation Study	Arnica, senescent leaf mass, 3rd and fourth combined
ARNSEN5	2020, Fumigation Study	Arnica, senescent fraction number 5 (g dry matter)
ARNSEN6	2021, Field Gradient Study	Arnica, biomass 6th senescent fraction 2021
ARNSEN7	2021, Field Gradient Study	Arnica, biomass 7th senescent fraction 2021
ARNSEN8	2021, Field Gradient Study	Arnica, biomass 8th senescent fraction 2021
ARNSENSUM	2020, Fumigation Study	Arnica, senescent leaves accumulated (g dry matter)
ARNSENSUM1bis5	2020, Fumigation Study	Arnica, senescent leaves accumulated in 2020, fractions 1 to 5 (g DM)
ARNSPAD0206	2020, Fumigation Study	Arnica, SPAD values, 2 June 2021
ARNSPAD0408	2020, Fumigation Study	Arnica, SPAD values, 4 August 2029
ARNSPAD0707	2020, Fumigation Study	Arnica, SPAD values, 7 July 2026
ARNSPAD0906	2020, Fumigation Study	Arnica, SPAD values, 9 June 2022
ARNSPAD1606	2020, Fumigation Study	Arnica, SPAD values, 16 June 2023
ARNSPAD2107	2020, Fumigation Study	Arnica, SPAD values, 21 July 2027
ARNSPAD2306	2020, Fumigation Study	Arnica, SPAD values, 23 June 2024
ARNSPAD2605	2020, Fumigation Study	Arnica, SPAD values, 26 May 2020
ARNSPAD2807	2020, Fumigation Study	Arnica, SPAD values, 28 July 2028
ARNSPAD3006	2020, Fumigation Study	Arnica, SPAD values, 30 June 2025
ARNTOT2021	2021, Field Gradient Study	Arnica, total biomass 2021
CONC	2020, Fumigation Study	Ammonia concentration (µg m-3)

FLON1	2020, Fumigation Study		Flower Number 25.03.2020
FLON2	2020, Fumigation Study		Flower Number 14.04.2020
FLON3	2020, Fumigation Study		Flower Number 14.05.2020
FLON4	2020, Fumigation Study		Flower Number 14.06.2020
FLONFINAL	2020, Fumigation Study		Flower Number at final harvest
HAR1DMC	2020, Fumigation Study		Intermediate Harvest dry matter content
HAR1DW	2020, Fumigation Study		Intermediate Harvest dry weight
HAR1FW	2020, Fumigation Study		Intermediate Harvest fresh weight
HEI1	2020, Fumigation Study		Height (cm) 25.03.2020
HEI2	2020, Fumigation Study		Height (cm) 14.04.2020
HEI3	2020, Fumigation Study		Height (cm) 14.05.2020
LEAFN1	2020, Fumigation Study		Leaf number at1st observation
LEAFN2	2020, Fumigation Study		Leaf number at 2nd observation
LEAFN3	2020, Fumigation Study		Leaf number at 3rd observation
MOLLEAFDM	2021, Field Gradient Study		Molinia, leaf dry matter (g)
MOLLENGTH	2021, Field Gradient Study		Molinia, length in cm
MOLROOTC13	2021, Field Gradient Study		Molinia root 13C, delta per mille
MOLROOTCN	2021, Field Gradient Study		Molinia, root CN
MOLROOTDM	2021, Field Gradient Study		Molinia, root dry matter (g)
MOLROOTN.	2021, Field Gradient Study		Molinia root N% dm
MOLROOTN15	2021, Field Gradient Study		Molinia root 15N, delta per mille
MOLRSR	2021, Field Gradient Study		Molinia, root-to-shoot-ratio
MOLSHOOTC	2021, Field Gradient Study		Molinia, shoot C concentration (%)
MOLSHOOTC13	2021, Field Gradient Study		Molinia shoot 13C, delta per mille
MOLSHOOTCN	2021, Field Gradient Study		Molinia, shoot CN
MOLSHOOTCPERCENT	2021, Field Gradient Study		Molinia, contribution of shoot to plant total (%)
MOLSHOOTN	2021, Field Gradient Study		Molinia, shoot N-concentration (%)
MOLSHOOTN15	2021, Field Gradient Study		Molinia shoot 15N, delta per mille
MOLSHOOTNPERC	2021, Field Gradient Study		Molinia, contribution of shoot N to plant total N (%)
MOLSTEMDM	2021, Field Gradient Study		Molinia, stem dry matter (g)
MOLSTEMNO	2021, Field Gradient Study		Molinia, number of stalks
MOLTOTAL21	2021, Field Gradient Study		Molinia, dry weght shoot plus root in 2021 (g)
MOLTOTAL2YEARS	2021, Field Gradient Study		Molinia, total dry mass both years (g)
MOLTOTALC21	2021, Field Gradient Study		Molinia, total C in g in 2021
MOLTOTALN21	2021, Field Gradient Study		Molinia, total N in g in 2021
NAME	2020 and 2021		Species Name
SEN1	2020, Fumigation Study		Senescent leaf mass, 1st
SEN2	2020, Fumigation Study		Senescent leaf mass, 2nd
SEN3	2020, Fumigation Study		Senescent leaf mass, 3nd
SEN4	2020, Fumigation Study		Senescent leaf mass, 4th
SEN5	2020, Fumigation Study		Senescent leaf mass, 5th, identical with ARNSEN5
SENPROCENT	2020, Fumigation Study		Senescent leave mass as percent of shoot
SENSUM	2020, Fumigation Study		Sum of senescent leaves
SET	2020 and 2021		CUT or UNCUT, plants subjected to an intermediate harvest (IMH) or not
SHOOT2020	2020, Fumigation Study		Total shoot mass fumigation study 2020, sum of IMH and final harvest
SHOOT2021	2021, Field Gradient Study		Total shoot mass field gradient study 2021
SHOOTN1	2020, Fumigation Study		Shoot Number June 2020
SHOOTN2	2020, Fumigation Study		Shoot Number August 2020
SPEC	2020 and 2021		Species Codes (Pot Numbers)
		1	Pulsatilla (1-100)
		2	Koeleria (101-200)
		3	Molinia (201-300)
		4	Arnica J (301-400)
		5	Arnica W (401-500)
		6	Antennaria (601-700)
		7	Carex (701-800)
		8	Dianthus d (801-900)
		9	Dianthus g (501-600)
		10	Erodium (901-950)
		11	Bromus (951-1000)
		12	Iberis (1001-1050)
		13	Trifolium (1051-1100)
		14	Legousia (1101-1150)
TEMP	2020, Fumigation Study		Mean Temperature (°C) in fumigation study
TOT1	2020, Fumigation Study		Total shoot mass intermediate harvest
TOT2	2020, Fumigation Study		Total shoot mass final harvest
TREAT	2020 and 2021		Ammonia treatments 1=Control
WAT	2020 and 2021		well watered or dry treated, WW or DT (33% reduced)

Annex 3

Results of the multiple comparisons (Tukey HSD tests) between the five ammonia treatments for each of the combinations water supply (well-watered or drought treated) and mowing (CUT in an intermediate harvest or UNCUT). Data are presented as mean values for all tested species and parameters. Abbreviations and parameters are explained in Annex 2 p. 168). Letters underneath each row of mean values indicate the results of pairwise comparisons, in which the use of the same letters indicates that there is no significant difference between the treatments. The bold numbers at the right side of each block indicate the grand mean for a parameter in each of the four treatment combinations.

Antennaria dioica

WAT	DT						WW						DT						WW					
SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
HAR1FW	1,2	1,1	1,1	1	0,8	**1,1**	2,5	2,3	1,7	2,6	2,1	**2,2**	NA	NA	NA	NA			NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1DW	0,4	0,4	0,5	0,4	0,3	**0,4**	0,7	0,7	0,5	0,8	0,7	**0,7**	NA	NA	NA	NA			NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1DMC	31	39	41	37	35	**37**	27	31	28	30	30	**29**	NA	NA	NA	NA			NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SHOOT2020	0,4	0,4	0,5	0,4	0,3	**0,4**	0,7	0,7	0,5	0,8	0,7	**0,7**	NA	NA	NA	NA			NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SHOOT2021	0,6	0,4	0,3	0,5	0,8	**0,5**	0,7	0,5	1	0,7	0,6	**0,7**	1,1	0,5	0,2	0,6	0,7	**0,6**	1,1	0,9	2,1	1,4	0,8	**1,3**
	a	ab	b	ab	ab		a	b	ab	ab	ab		a	ab	b	ab	ab		ab	ab	a	ab	b	

***Arnica montana* "Jelitto"**

WAT	DT						WW						DT						WW					
SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
SEN1	0,9	0,9	0,6	0,7	1,2	**0,8**	0,7	0,8	0,5	0,9	0,7	**0,7**	0,9	0,8	0,5	1,4	0,7	**0,9**	0,5	0,4	0,6	0,4	0,9	**0,6**
	a	a	a	a	a		a	a	a	a	a		ab	ab	b	a	ab		a	a	a	a	a	
SEN2	0,4	0,5	0,7	0,6	0,4	**0,5**	1	0,7	0,2	1,2	1	**0,8**	0,5	0,5	0,8	0,3	0,6	**0,5**	0,7	0,3	0,4	0,4	1,1	**0,6**
	a	a	a	a	a		a	a	a	a	a		ab	ab	b	a	ab		a	a	a	a	a	
SEN3	0,8	0,5	0,8	0,5	0,4	**0,6**	1	0,9	1	0,8	0,9	**0,9**	0,5	0,6	0,5	0,1	0,4	**0,4**	1,1	0,9	1,1	1,3	0,7	**1**

	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN4	1,1	0,2	0,7	0,6	0,9	**0,7**	1,2	0,8	0,5	0,6	0,3	**0,7**	0,5	0,4	0,3	1,1	0,8	**0,6**	0,8	0,6	0,5	0,6	0,4	**0,6**
	a	a	a	a	a		a	ab	ab	ab	b		a	a	a	a	a		a	a	a	a	a	
SENSUM	3,2	2,1	2,8	2,4	2,9	**2,7**	3,9	3,2	2,3	3,5	3	**3,2**	2,4	2,3	2,2	2,8	2,5	**2,4**	3,1	2,2	2,6	2,7	3,1	**2,8**
	a	a	a	a	a		a	ab	ab	ab	b		a	a	a	a	a		a	a	a	a	a	
LEAFN1	14	21	20	19	15	**18**	16	23	18	21	20	**20**	15	10	13	15	14	**13**	11	20	20	15	13	**16**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
LEAFN2	18	26	29	24	26	**25**	21	27	24	28	28	**26**	20	17	17	21	18	**19**	15	22	24	20	23	**21**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
LEAFN3	24	29	40	37	32	**33**	32	37	33	43	37	**37**	25	22	23	33	26	**26**	26	27	36	29	33	**30**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOTN1	3,4	4,8	5,8	5	4,6	**4,7**	5,2	4,8	3,8	4,8	5,6	**4,8**	3	3,8	3,4	3,6	3	**3,4**	3,4	3,6	4,4	4,6	4,2	**4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOT2020	3,2	2,2	3	2,4	3	**2,8**	4,5	3,6	2,7	3,7	3,2	**3,5**	2,5	2,3	2,4	2,9	2,8	**2,6**	3,7	2,6	3,6	3,2	3,7	**3,4**
	a	a	a	a	a		a	ab	ab	ab	b		a	a	a	a	a		a	a	a	a	a	
SHOOT2021	1,3	0,9	1,1	NA	3,8	**1,8**	4	3,2	4,1	1,9	4,4	**3,5**	NA	NA	1,6	4	3,9	**3,2**	3,8	3,7	4,3	3,3	5,3	**4,1**
	a	a	a		a		a	a	a	a	a				a	a	a		a	a	a	a	a	
ARNSEN5	0	0,1	0,2	0,1	0,2	**0,1**	0,6	0,5	0,5	0,2	0,2	**0,4**	0	0	0,2	0,1	0,3	**0,1**	0,6	0,4	1	0,5	0,6	**0,6**
	a	a	a	a	a		a	a	a	a	a		b	ab	ab	ab	a		a	a	a	a	a	
ARNSENSUM	3,2	2,1	2,8	2,4	2,9	**2,7**	3,9	3,2	2,3	3,5	3	**3,2**	2,4	2,3	2,2	2,8	2,5	**2,4**	3,1	2,2	2,6	2,7	3,1	**2,8**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNSENSUM1bis5	3,2	2,2	3	2,4	3	**2,8**	4,5	3,6	2,7	3,7	3,2	**3,5**	2,5	2,3	2,4	2,9	2,8	**2,6**	3,7	2,6	3,6	3,2	3,7	**3,4**
	a	a	a	a	a		a	ab	b	ab	ab		a	a	a	a	a		a	a	a	a	a	
ARNSEN12	1,2	1,4	1,3	1,3	1,5	**1,3**	1,7	1,4	0,8	2	1,8	**1,5**	1,4	1,3	1,3	1,6	1,3	**1,4**	1,1	0,7	1	0,8	2	**1,1**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		ab	b	ab	ab	a	
ARNSEN34	1,9	0,8	1,5	1	1,3	**1,3**	2,2	1,7	1,5	1,5	1,2	**1,6**	1	1	0,9	1,2	1,2	**1**	1,9	1,5	1,6	1,9	1,1	**1,6**
	a	b	ab	ab	ab		ab	ab	ab	ab	b		a	a	a	a	a		a	a	a	a	a	
ARNN.12	1,4	1,6	1,5	1,5	1,4	**1,5**	1,4	1,4	1,3	1,6	1,6	**1,4**	1,7	1,8	1,4	1,5	1,4	**1,6**	1,2	1,5	1,2	1,3	1,5	**1,3**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN.34	2	2,6	2,1	2,4	2,4	**2,3**	1,5	1,6	1,5	1,6	2,5	**1,8**	2,8	2,8	2,1	2	2,1	**2,4**	1,4	1,7	1,2	1,4	2	**1,5**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN1512	85	89	95	95	86	**90**	74	81	75	92	79	**80**	91	89	92	104	89	**93**	80	77	85	85	76	**81**
	a	a	a	a	a		b	ab	ab	a	ab		a	a	a	a	a		a	a	a	a	a	
ARNN1534	47	48	60	62	51	**54**	46	60	68	53	56	**56**	53	54	56	46	51	**52**	69	63	68	68	46	**63**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	ab	a	ab	b	
ARNNTOT	55	42	52	43	55	**50**	58	48	32	56	56	**50**	49	50	37	48	45	**46**	41	34	32	36	52	**39**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNCN12	26	27	26	27	27	**27**	28	28	30	23	25	**27**	24	21	27	25	27	**25**	30	26	31	30	25	**28**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	

ARNCN34	19	17	18	17	17	**18**	25	26	26	24	16	**23**	14	13	18	19	18	**16**	31	24	32	30	19	**27**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN.5	1,9	1,4	2,2	1,8	1,5	**1,8**	1,3	1,6	1,2	1,5	2,1	**1,6**	1,3	2,9	1,7	2,1	1,5	**1,9**	1,3	1,5	1,2	1,4	1,1	**1,3**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN155	35	25	36	41	34	**34**	26	34	31	33	35	**32**	26	42	37	39	34	**36**	34	29	37	36	29	**33**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNCN5	19	18	13	15	21	**17**	24	21	27	18	17	**21**	24	12	14	14	22	**17**	28	19	26	22	28	**25**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	

***Arnica montana* "Weiherberg"**

WAT	DT						WW						DT						WW					
SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
SEN1	0,5	0,5	0,3	0,5	0,4	**0,5**	0,5	0,3	0,6	0,3	0,4	**0,4**	0,4	0,5	0,5	0,7	0,8	**0,6**	0,4	0,5	0,5	0,4	0,5	**0,4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN2	0,5	0,6	0,5	0,4	0,4	**0,5**	0,6	0,2	0,3	0,4	0,5	**0,4**	0,4	0,4	0,6	0,5	0,3	**0,5**	0,4	0,4	0,4	0,2	0,5	**0,4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN3	0,2	0,3	0,5	0,4	0,4	**0,4**	0,4	0,6	0,5	0,8	0,4	**0,6**	0,5	0,4	0,4	0,2	0,4	**0,4**	0,6	1	0,7	1	0,6	**0,8**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN4	0,2	0,5	0,3	0,5	0,3	**0,4**	0,5	0,3	0,3	0,3	0,2	**0,3**	0,5	0,2	0,3	0,6	0,4	**0,4**	0,5	0,8	0,3	0,4	0,3	**0,5**
	a	a	a	a	a		a	ab	ab	ab	b		a	a	a	a	a		a	a	a	a	a	
SENSUM	1,4	1,9	1,6	1,8	1,6	**1,7**	2	1,4	1,7	1,8	1,4	**1,7**	1,8	1,6	1,9	2,1	1,9	**1,8**	1,8	2,7	1,8	2	1,9	**2**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
LEAFN1	7,2	9,4	6,4	6,2	11	**8**	8	7,2	7,6	7,2	6	**7,2**	7,2	6,6	8	7,2	7,4	**7,3**	7,6	8,4	7,2	6,4	7,4	**7,4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
LEAFN2	10	10	9,4	7,6	12	**9,8**	8,6	7,6	10	9,2	8,2	**8,8**	7,8	8,2	8,4	8	9,2	**8,3**	9,2	11	8	8	8	**8,9**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
LEAFN3	16	16	17	14	19	**16**	15	13	18	18	16	**16**	11	16	15	16	17	**15**	18	22	15	17	17	**18**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOTN1	1,8	1,8	1,8	1	2,4	**1,8**	1,4	1	1,6	1,6	1,4	**1,4**	1,2	1,2	1	1,4	2	**1,4**	1,4	2	1,2	1	1,4	**1,4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOT2020	1,4	2,3	1,8	1,9	1,6	**1,8**	2,2	1,5	2,2	2,4	2,5	**2,2**	1,8	1,8	2,1	2,2	2,1	**2**	2,2	3,2	2,1	2,8	1,9	**2,5**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOT2021	NA	2,1	NA	NA	NA	**2,1**	3	NA	3,6	1,7	2	**2,6**	NA	2,7	1,7	0,7	4,5	**2,4**	1,9	3,4	3,1	2,2	2,4	**2,6**
		a					a		a	a	a			a	a	a	a		a	a	a	a	a	
ARNSEN5	0	0,4	0,2	0,1	0,1	**0,2**	0,3	0,1	0,5	0,6	0,4	**0,4**	0	0,4	0,2	0,2	0,2	**0,2**	0,4	0,6	0,3	0,8	0	**0,4**

	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNSENSUM	1,4	1,9	1,6	1,8	1,6	**1,7**	2	1,4	1,7	1,8	1,4	**1,7**	1,8	1,6	1,9	2,1	1,9	**1,8**	1,8	2,7	1,8	2	1,9	**2**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNSENSUM1bis5	1,4	2,3	1,8	1,9	1,6	**1,8**	2,2	1,5	2,2	2,4	1,8	**2**	1,8	2	2,1	2,2	2,1	**2**	2,2	3,2	2,1	2,8	1,9	**2,5**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		ab	a	ab	ab	b	
ARNSEN12	1	1,1	0,8	0,8	0,8	**0,9**	1	0,5	0,9	0,7	0,8	**0,8**	0,8	1	1,1	1,2	1,1	**1**	0,7	0,8	0,8	0,6	1	**0,8**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNSEN34	0,4	0,8	0,8	1	0,8	**0,8**	0,9	0,9	0,8	1,1	0,7	**0,9**	1	0,6	0,8	0,9	0,8	**0,8**	1,1	1,8	1	1,4	0,9	**1,3**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN.12	1,9	1,4	1,5	1,4	1,5	**1,5**	1,3	1,1	1,3	1,4	1,7	**1,4**	1,6	1,7	1,6	1,4	1,5	**1,5**	1,2	1,2	0,9	1,2	1,7	**1,3**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN.34	3,3	1,8	2,3	2,2	2,6	**2,4**	2,3	2,3	1,9	1,3	1,7	**1,9**	2,5	2,3	2,4	2,3	2,4	**2,4**	2,2	1,4	1,1	1,3	2,4	**1,7**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN1512	81	93	103	102	103	**96**	84	86	89	90	89	**88**	79	91	102	102	110	**97**	86	93	91	87	85	**88**
	b	ab	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN1534	70	59	71	69	71	**68**	56	70	70	72	66	**67**	57	67	63	57	68	**63**	64	69	72	71	63	**68**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNNTOT	33	28	29	32	30	**30**	34	26	23	25	25	**27**	35	31	35	33	35	**34**	30	36	19	25	37	**30**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNCN12	20	27	25	28	26	**25**	29	29	30	26	22	**27**	24	25	25	28	26	**26**	30	30	38	30	22	**30**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		ab	ab	ab	ab	a	
ARNCN34	10	21	19	19	16	**17**	19	18	24	31	22	**23**	16	19	16	19	18	**17**	20	27	33	30	16	**25**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		ab	ab	a	ab	b	
ARNN.5	NA	1,7	2,1	1,9	2,4	**2**	1,6	1,5	1,4	1,3	1,8	**1,5**	2,6	1,3	2	1,6	1,6	**1,8**	1,4	1,5	1,2	1,5	1,2	**1,4**
		a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
ARNN155	NA	35	39	43	57	**44**	35	38	40	29	35	**35**	52	37	47	36	44	**43**	34	35	40	33	17	**32**
		a	a	a	a		a	a	a	a	a		a	a	a	a	a		ab	ab	a	ab	b	
ARNCN5	NA	17	14	17	9,8	**14**	20	17	21	21	19	**20**	11	25	16	17	21	**18**	19	22	31	20	26	**23**
		a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	

Carex arenaria

WAT	DT						WW						DT						WW					
SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
HEI1	9,8	10	11	9,5	11	**10**	11	9,5	11	9	10	**10**	9,3	11	11	9,9	11	**10**	12	9,3	10	11	11	**11**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	

HEI2	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA		19	21	18	18	15	**18**	25	20	19	22	27	**23**
													a	a	a	a	a		a	a	a	a	a	
SHOOTN1	12	10	10	12	9	**11**	13	13	14	13	13	**13**	12	12	12	13	8,2	**11**	12	14	14	13	10	**13**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOTN2	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA		20	25	23	23	13	**21**	26	30	26	29	22	**27**
													ab	a	ab	ab	b		a	a	a	a	a	
HAR1FW	1,3	2,1	1,7	0,9	0,6	**1,3**	3,5	2,4	2,9	3,1	3,3	**3**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1DW	0,5	0,7	0,6	0,4	0,3	**0,5**	1,2	0,8	0,9	1	1	**1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	ab	ab	ab	b	b		a	b	ab	ab	ab													
HAR1DMC	36	33	32	59	68	**45**	35	32	33	33	32	**33**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
TOT1	0,5	0,7	0,4	0,4	0,3	**0,5**	1,2	0,8	0,9	1	1	**1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	ab	ab	ab	b	b		a	b	ab	ab	ab													
TOT2	1	0,5	0,9	0,8	0,7	**0,8**	1,3	1,1	1,1	1,1	1,2	**1,1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	b	ab	ab	ab		a	a	a	a	a													
SHOOT2020	1,5	1,2	1,4	1,2	1	**1,3**	2,5	1,8	2	2,1	2,2	**2,1**	1,5	1,4	1,2	1,2	0,8	**1,2**	2,4	1,9	1,9	1,9	2,2	**2,1**
	a	ab	ab	ab	b		a	b	ab	ab	ab		a	a	a	a	a		a	a	a	a	a	
SHOOT2021	2,9	3	3	3	2,7	**2,9**	2,7	2,9	2,7	1,8	2,8	**2,6**	3,7	3,2	3,4	2,9	2,7	**3,2**	3,7	3,4	3,9	2,8	3,3	**3,4**
	a	a	a	a	a		a	ab	ab	b	ab		a	a	a	a	a		a	a	a	a	a	

Dianthus deltoides

WAT	DT						WW						DT						WW					
SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
FLON1	0,2	0,4	0,6	2	1,2	**0,9**	0,8	0,2	1,4	0,8	1	**0,8**	1,4	0,6	1,6	1	0,6	**1**	2,2	3	1,6	2,4	2,4	**2,3**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
FLON2	9,2	10	19	13	13	**13**	27	26	22	26	18	**24**	13	14	15	15	11	**13**	27	25	27	18	27	**25**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN1	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA		0,4	0,5	1	1	0,8	**0,8**	0,9	0,7	0,6	0,5	0,8	**0,7**
													a	a	a	a	a		a	a	a	a	a	
HAR1FW	2,6	3,9	1,8	1,8	1,7	**2,4**	4,1	4,2	3,7	3,9	3	**3,8**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	ab	a	b	b	b		a	a	a	a	a													
HAR1DW	0,7	1,1	0,6	0,6	0,6	**0,7**	1,2	1,3	1,1	1,2	0,9	**1,1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	ab	a	b	ab	b		a	a	a	a	a													
HAR1DMC	30	29	35	38	38	**34**	30	30	30	31	30	**30**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	

	a	a	a	a	a		a	a	a	a	a													
TOT1	0,7	1,1	0,6	0,6	0,6	**0,7**	1,2	1,3	1,1	1,2	0,9	**1,1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	ab	a	b	ab	b		a	a	a	a	a													
TOT2	0,8	0,8	0,4	0,7	0,5	**0,7**	0,9	0,4	0,5	0,7	1,1	**0,7**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SHOOT2020	1,5	1,9	1	1,4	1,1	**1,4**	2,1	1,7	1,6	1,9	2	**1,9**	1,8	1,5	1,5	1,3	1,1	**1,5**	2,6	2	2	1,8	2,3	**2,1**
	ab	a	b	ab	b		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOT2021	1,2	1,1	0,7	0,9	1,1	**1**	1,2	1,2	1	1,1	1	**1,1**	1,3	1,1	1,3	1,7	0,6	**1,2**	0,9	1,3	1,1	1,1	0,7	**1**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	

Koeleria glauca

WAT	DT						WW						DT						WW					
SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
SEN1	0,9	0,6	0,5	0,9	0,6	**0,7**	0,6	0,6	0,5	0,6	0,8	**0,6**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1FW	4,1	4	4	2,9	4,3	**3,9**	6,7	5,5	4,1	5,5	5,1	**5,4**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	ab	b	ab	ab													
HAR1DW	1,5	1,4	1,5	1,2	1,7	**1,4**	2,2	2	1,5	2,1	1,9	**1,9**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1DMC	38	36	37	44	39	**39**	33	37	37	39	37	**36**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
TOT1	2,4	2	2	2,1	2,3	**2,2**	2,8	2,6	2,1	2,7	2,7	**2,6**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SENPROCENT	38	29	26	44	27	**33**	20	24	24	21	30	**24**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		b	ab	ab	ab	a													
TOT2	0,7	0,5	0,7	0,6	0,7	**0,6**	1	0,9	0,7	0,7	0,9	**0,8**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SHOOT2020	3,1	2,5	2,7	2,6	3	**2,8**	3,7	3,5	2,7	3,3	3,5	**3,4**	2,8	1,6	1,8	2,1	2	**2,1**	3,2	2,5	2	2,6	2,8	**2,6**
	a	a	a	a	a		a	a	a	a	a		a	b	ab	ab	ab		a	ab	b	ab	ab	
SHOOT2021	0,8	0,6	0,8	0,7	0,8	**0,7**	0,7	0,8	0,7	0,6	0,8	**0,7**	1,1	0,6	0,9	1	0,8	**0,9**	1,2	1	0,8	1,1	0,9	**1**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	

Molinia caerulea

WAT	DT						WW						DT						WW					

SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
FLON1	1,4	1,2	1,6	0,8	0,4	**1,1**	0,2	1,2	0,8	0,2	1	**0,7**	1,2	0,8	0,8	0,8	2	**1,1**	0	2,4	2,2	1,8	2,4	**1,8**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
FLON2	1,4	1,4	1,6	1	0,6	**1,2**	0,2	1,2	1,2	0,4	1,2	**0,8**	1,2	1,4	0,8	1,4	2,6	**1,5**	0	2,4	2,4	2	2,8	**1,9**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
FLON3	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA		1,2	1,4	1	1,4	3		3,4	2,4	2,4	2	4	
													a	a	a	a	a		a	a	a	a	a	
FLON4	0,8	0,6	0,4	0	0,8	**0,5**	3,6	0,6	3,2	0,4	0,6	**1,7**	1,2	1,4	1,4	1,4	3,4	**1,8**	3,4	2,2	2,4	2	4,8	**3**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
FLONFINAL	1,4	0,8	1,4	1,2	0,4	**1**	1	1,2	1,2	1,6	2	**1,4**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SEN1	0,1	0,1	0,1	0,1	0,1	**0,1**	0,1	0,1	0,1	0,1	0,1	**0,1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SHOOTN1	24	25	26	21	26	**24**	25	27	24	26	25	**25**	29	23	24	21	26	**25**	23	23	21	22	20	**22**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOTN2	27	30	27	26	29	**28**	29	30	29	32	30	**30**	27	30	28	26	26	**27**	28	26	25	29	27	**27**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
HAR1FW	1,7	2,1	1,8	1,8	1,8	**1,8**	3,2	1,9	2,5	2,7	3,2	**2,7**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		ab	b	ab	ab	a													
HAR1DW	0,6	0,8	0,7	0,7	0,6	**0,7**	1,1	0,7	0,9	1	1,2	**1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1DMC	38	37	37	38	36	**37**	35	36	35	37	36	**36**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
TOT1	0,7	0,9	0,8	0,8	0,8	**0,8**	1,2	0,8	1	1,1	1,3	**1,1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		ab	b	ab	ab	a													
SENPROCENT	13	14	11	12	17	**13**	6	8,5	13	12	9,6	**9,8**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
TOT2	0,5	0,4	0,5	0,6	0,5	**0,5**	0,6	0,5	0,6	0,6	0,6	**0,6**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SHOOT2020	1,2	1,4	1,3	1,3	1,3	**1,3**	1,8	1,2	1,7	1,7	1,9	**1,7**	1	1	1	1	0,8	**1**	1,3	0,9	1	1,1	1,4	**1,2**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		ab	b	b	b	a	
SHOOT2021	2	1,7	2,9	2,8	3,1	**2,5**	1,3	2,4	2	1,6	1,8	**1,8**	2,5	2,7	2,8	3,2	3,5	**2,9**	2,1	2,8	3,2	3,4	3,4	**3**
	bc	c	ab	ab	a		a	a	a	a	a		a	a	a	a	a		b	ab	a	a	a	
MOLROOTDM	13	14	15	15	13	**14**	16	15	14	14	14	**15**	16	15	16	14	15	**15**	16	16	18	17	17	**17**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLROOTC13	-28	-28	-28	-28	-28	**-28**	-29	-28	-28	-29	-28	**-28**	-28	-28	-28	-28	-28	**-28**	-28	-28	-28	-28	-28	**-28**

	a	a	a	a	a		ab	ab	a	b	ab		a	a	a	a	a		a	a	a	a	a	
MOLROOTN	0,5	0,4	0,4	0,4	0,5	**0,4**	0,4	0,4	0,4	0,4	0,5	**0,4**	0,5	0,5	0,4	0,5	0,5	**0,5**	0,4	0,4	0,4	0,4	0,5	**0,4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLROOTN15	65	69	68	71	67	**68**	62	66	63	70	64	**65**	67	66	68	71	68	**68**	62	77	71	69	61	**68**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLROOTCN	92	102	103	101	93	**98**	96	99	103	110	91	**100**	85	90	104	96	85	**92**	98	101	96	102	89	**97**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLSHOOTCPERCENT	48	48	48	48	48	**48**	46	48	48	47	48	**47**	48	49	48	48	48	**48**	48	48	49	48	48	**48**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLSHOOTC13	-29	-29	-29	-29	-29	**-29**	-29	-29	-29	-29	-29	**-29**	-29	-29	-29	-28	-29	**-29**	-29	-29	-28	-29	-29	**-29**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLSHOOTNPERC	0,4	0,5	0,4	0,4	0,5	**0,4**	0,5	0,4	0,4	0,4	0,4	**0,4**	0,4	0,5	0,5	0,3	0,4	**0,4**	0,5	0,3	0,4	0,3	0,4	**0,4**
	a	a	a	a	a		a	b	ab	ab	ab		a	a	a	a	a		a	b	ab	ab	ab	
MOLSHOOTN15	59	62	52	63	49	**57**	54	61	52	62	50	**56**	60	60	57	66	55	**60**	53	74	61	70	46	**61**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		ab	a	ab	ab	b	
MOLSHOOTCN	130	104	118	125	105	**117**	90	127	111	115	118	**112**	121	108	106	140	111	**117**	104	157	130	146	129	**133**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLLENGTH	39	34	45	43	48	**42**	36	42	38	34	45	**39**	43	40	44	50	48	**45**	41	44	51	43	46	**45**
	ab	b	a	ab	a		a	a	a	a	a		a	a	a	a	a		b	ab	a	ab	ab	
MOLSTEMNO	13	13	18	18	17	**16**	9,6	18	14	9,4	13	**13**	16	20	17	17	19	**18**	16	19	18	21	22	**19**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLLEAFDM	0,9	0,8	1,1	1,2	1,3	**1,1**	0,7	1,1	0,8	0,9	0,7	**0,8**	1	1,1	1,1	1,2	1,2	**1,1**	0,9	0,9	1,1	1,5	1	**1,1**
	b	b	ab	ab	a		a	a	a	a	a		a	a	a	a	a		b	b	ab	a	ab	
MOLSTEMDM	1,1	0,9	1,8	1,6	1,8	**1,4**	0,6	1,3	1,1	0,7	1,1	**1**	1,5	1,7	1,7	2	2,3	**1,8**	1,2	1,8	2,1	2,1	2,3	**1,9**
	ab	b	a	ab	a		a	a	a	a	a		a	a	a	a	a		b	ab	a	a	a	
MOLTOTAL21	15	15	18	17	16	**16**	17	18	16	16	15	**16**	18	18	19	17	18	**18**	18	19	22	21	20	**20**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLSHOOTN	7,5	7,7	12	11	14	**11**	6,6	9	8,6	6,5	7,3	**7,6**	10	13	13	11	15	**12**	9,9	8,6	12	12	13	**11**
	b	b	ab	ab	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLSHOOTC	1	0,8	1,4	1,3	1,5	**1,2**	0,6	1,1	0,9	0,8	0,9	**0,9**	1,2	1,3	1,3	1,5	1,7	**1,4**	1	1,3	1,6	1,7	1,6	**1,4**
	bc	c	ab	ab	a		a	a	a	a	a		a	a	a	a	a		b	ab	a	a	a	
MOLTOTALN21	66	66	76	73	73	**71**	75	74	69	63	72	**71**	86	88	80	73	87	**83**	77	77	92	86	91	**84**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLTOTALC21	13	15	17	16	13	**15**	15	16	16	17	13	**16**	15	15	18	14	14	**15**	16	18	19	20	17	**18**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
MOLRSR	6,6	8,4	5,1	5,3	4,2	**5,9**	13	6,5	7,8	9,8	7,9	**8,9**	6,5	5,9	5,9	4,3	4,2	**5,4**	7,5	5,9	5,8	4,9	5	**5,8**
	ab	a	bc	bc	c		a	bc	ab	ab	ab		a	ab	ab	b	b		a	b	a	a	a	

Pulsatilla vulgaris

WAT	DT						WW						DT						WW					
SET	CUT						CUT						UNCUT						UNCUT					
TREAT	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
CONC	5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19		5,6	4,6	5,7	8,6	19	
HEI1	10	15	14	13	13	**13**	12	13	8,1	13	14	**12**	10	10	8,4	14	14	**11**	9,9	15	11	14	15	**13**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
HEI2	17	22	25	21	24	**22**	26	30	17	21	21	**23**	21	22	16	25	21	**21**	22	23	16	23	21	**21**
	a	a	a	a	a		ab	a	b	ab	ab		a	a	a	a	a		a	a	a	a	a	
HEI3	15	15	24	20	23	**19**	24	26	18	15	20	**21**	23	21	9,4	25	19	**20**	21	20	14	21	20	**19**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
FLON1	1,4	1,6	2,2	2,2	2,6	**2**	1,6	1,8	0,8	1,8	2,2	**1,6**	1,4	1,4	2,4	2,6	2,6	**2,1**	1,2	1,8	1,4	1,4	2,8	**1,7**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
FLON2	2,2	2,4	2,4	2,6	2,8	**2,5**	1,8	2,6	2,8	2,8	2,4	**2,5**	1,8	2	2,8	3,2	3	**2,6**	1,6	3,4	2,8	3,4	2,8	**2,8**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN1	0,5	0,9	0,9	1	1,2	**0,9**	0,4	1	1	0,7	0,9	**0,8**	1,3	1,1	1	1,2	1	**1,1**	0,5	0,9	0,6	0,9	0,9	**0,8**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN2	0,4	0,3	0,3	0,5	0,4	**0,4**	0,5	0,3	0,3	0,4	0,4	**0,4**	0,7	0,5	0,5	0,4	0,4	**0,5**	0,5	0,4	0,5	0,4	0,4	**0,4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SEN3	0,1	0,2	0,1	0,2	0,2	**0,2**	0,1	0,3	0,1	0,1	0,1	**0,2**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SEN4	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA		0,4	0,6	0,9	0,9	0,9	**0,7**	0,4	0,2	0,3	0,2	0,3	**0,3**
													a	a	a	a	a		a	a	a	a	a	
SEN5	0,3	0,3	0,3	0,3	0,5	**0,3**	0,6	0,4	0,4	0,5	0,8	**0,6**	0,9	0,8	0,6	1	0,9	**0,9**	1,4	1,5	1,4	1,4	1,4	**1,4**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SENSUM	1,1	1,4	1,3	1,7	1,7	**1,4**	0,9	1,7	1,5	1,2	1,4	**1,3**	2	1,6	1,3	1,6	1,4	**1,6**	0,9	1,3	1,1	1,4	1,3	**1,2**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
HAR1FW	4,4	4,3	4,3	4	4,2	**4,2**	6,6	5	5,4	6,6	6,3	**6**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1DW	1,3	1,4	1,3	1,3	1,3	**1,3**	1,9	1,5	1,6	1,9	1,8	**1,7**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
HAR1DMC	30	33	31	31	30	**31**	29	29	29	29	29	**29**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
TOT1	2,4	2,8	2,6	3	3	**2,7**	2,9	3,1	3,1	3,1	3,2	**3,1**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
SENPROCENT	44	48	48	57	55	**50**	33	54	50	37	43	**43**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
	a	a	a	a	a		a	a	a	a	a													
TOT2	0,3	0,3	0,3	0,3	0,5	**0,3**	0,6	0,5	0,4	0,5	0,8	**0,6**	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	

	a	a	a	a	a		a	a	a	a	a													
SHOOT2020	2,7	3,1	2,9	3,3	3,5	**3,1**	3,5	3,3	3,5	3,6	4	**3,6**	3,4	3	3,2	3,5	3,3	**3,3**	2,7	3	2,8	3	3	**2,9**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	
SHOOT2021	1,6	1,3	1,7	1,7	1,6	**1,6**	1,6	1,6	1,9	1,9	1,5	**1,7**	2,1	1,8	1,6	1,4	1,6	**1,7**	1,8	1,8	2	1,6	1,8	**1,8**
	a	a	a	a	a		a	a	a	a	a		a	a	a	a	a		a	a	a	a	a	

Species only used in the field gradient study

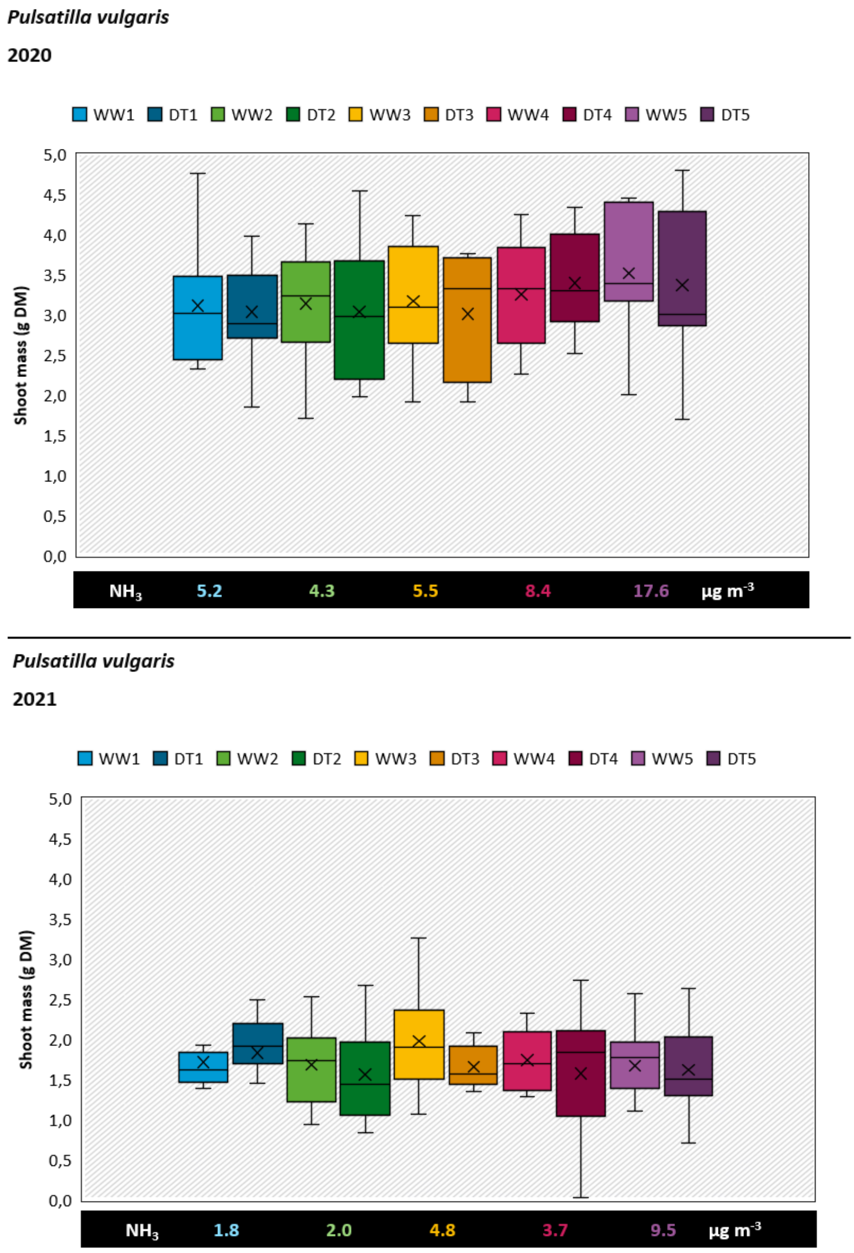
				SHOOT2021				
TREAT				1	2	3	4	5
Dianthus gratianopoilitanus				6,3	7,3	7,3	6,5	5,8
				a	a	a	a	a
Bromus hordeaceus				0,3	0,4	0,4	0,4	0,5
				b	b	b	b	a
Erodium cicutarium				0,2	0,2	0,3	0,2	0,3
				ab	b	ab	ab	a
Iberis amara				0,4	0,4	0,4	0,4	0,5
				ab	b	b	ab	a
Legousia speculum-veneris				0,1	0,1	0,1	0,1	0,1
				a	a	a	a	a
Trifolium arvense				1,4	1,6	1,5	1,6	0,9
				a	a	a	a	a

Annex 4

Figure 37: Growth of perennial plant species exposed to ammonia and drought in the years 2020 (above) and 2021 (below)

Box-and whisker plots refer to the eight plant species *Pulsatilla vulgaris*, *Koeleria glauca*, *Molinia caerulea*, *Antennaria dioica*, *Arnica montana* (Jelitto), *Arnica montana* (Weiherberg), *Dianthus deltoides* and *Carex arenaria* (a -h). Ten replicates each were grown under well-watered conditions (WW) and in dry treatments (DT), darker boxes. Graphs do not differentiate between plants that were subjected to an intermediate and a final harvest and those that were only harvested at the end of the season. “x” refers to the average. Shoot masses were harvested at the end of the season (for dates refer to Table 14).

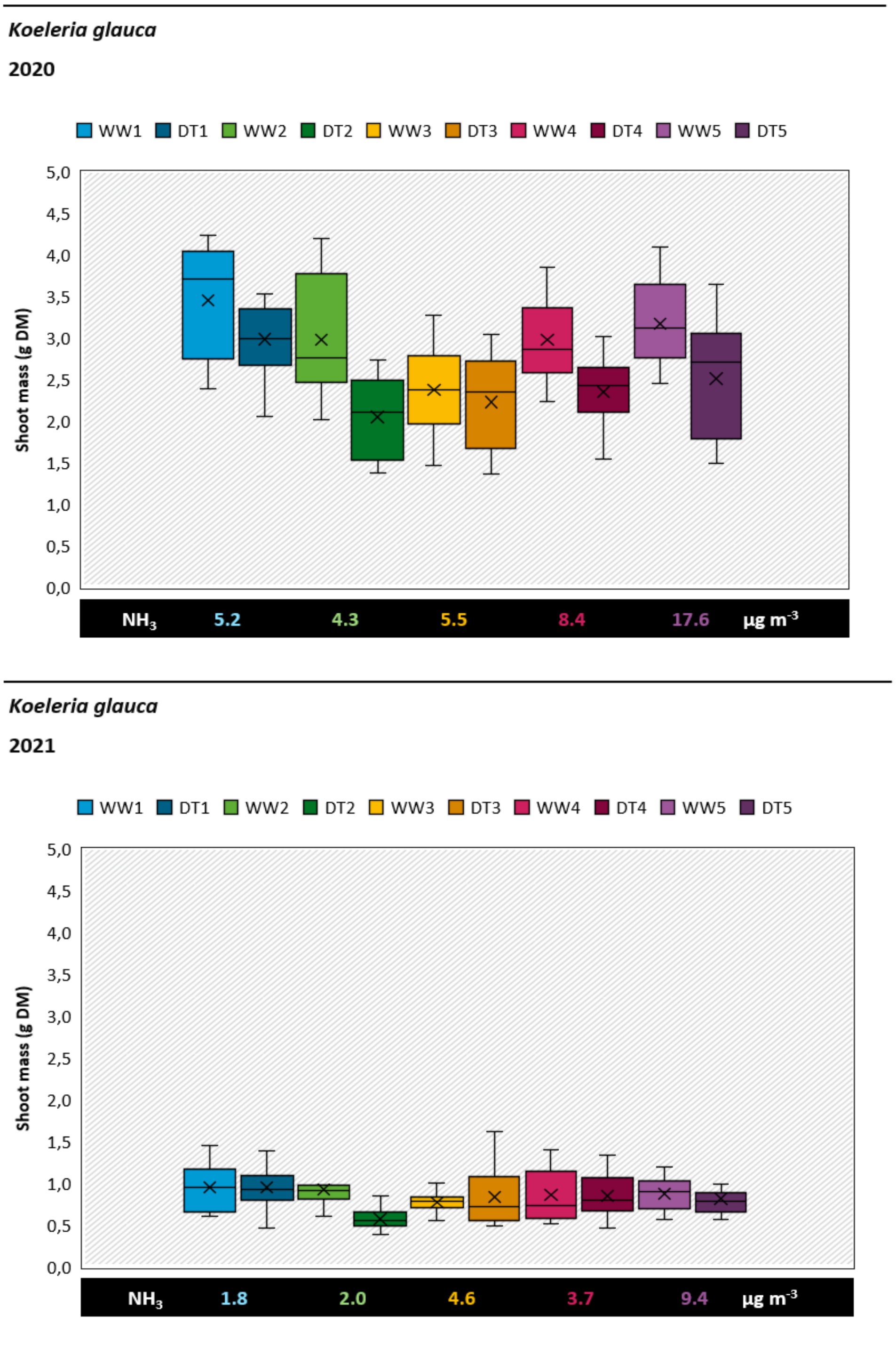
37a)



Source: Own illustration, University of Hohenheim.

Continued:

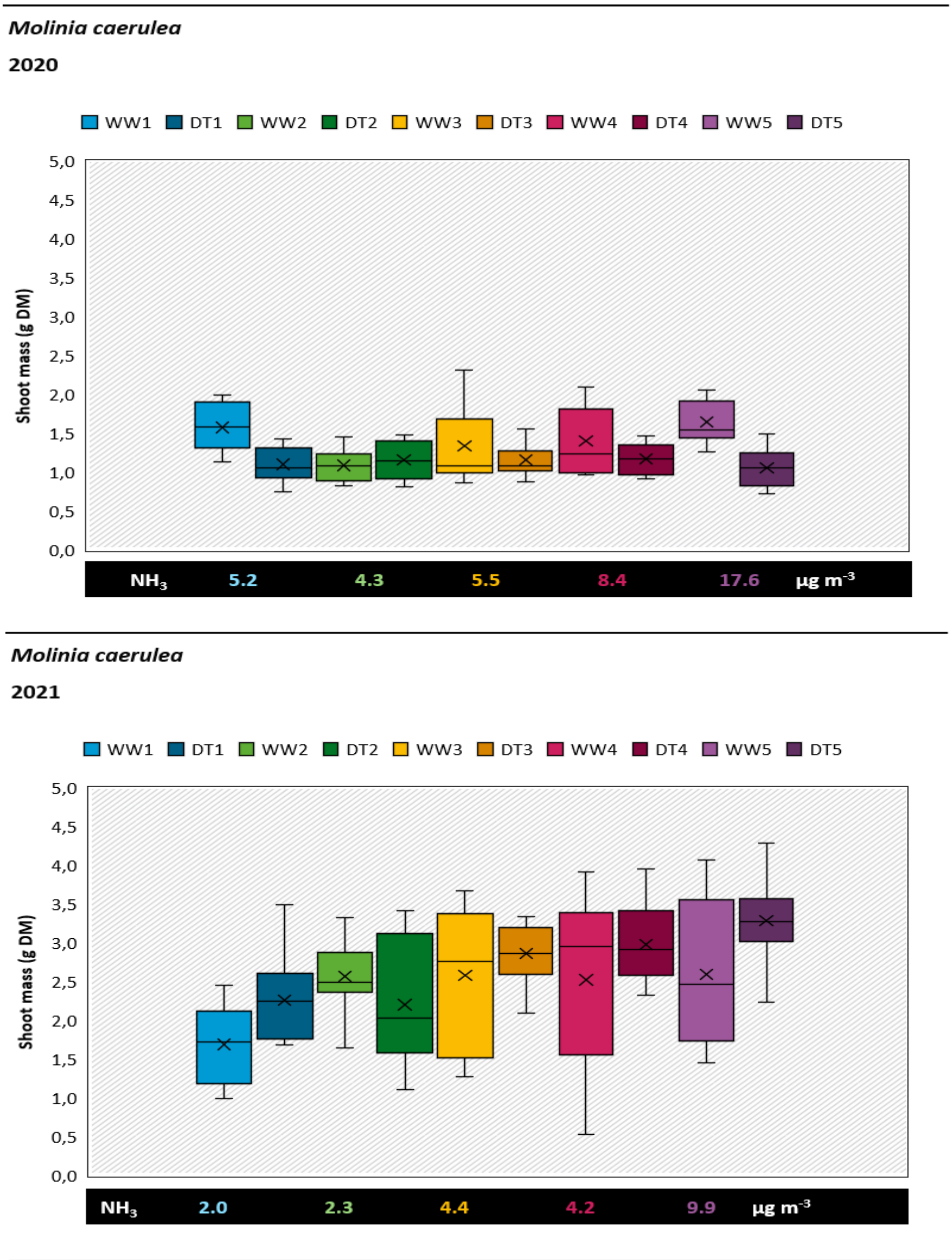
37b)



Source: Own illustration, University of Hohenheim.

Continued:

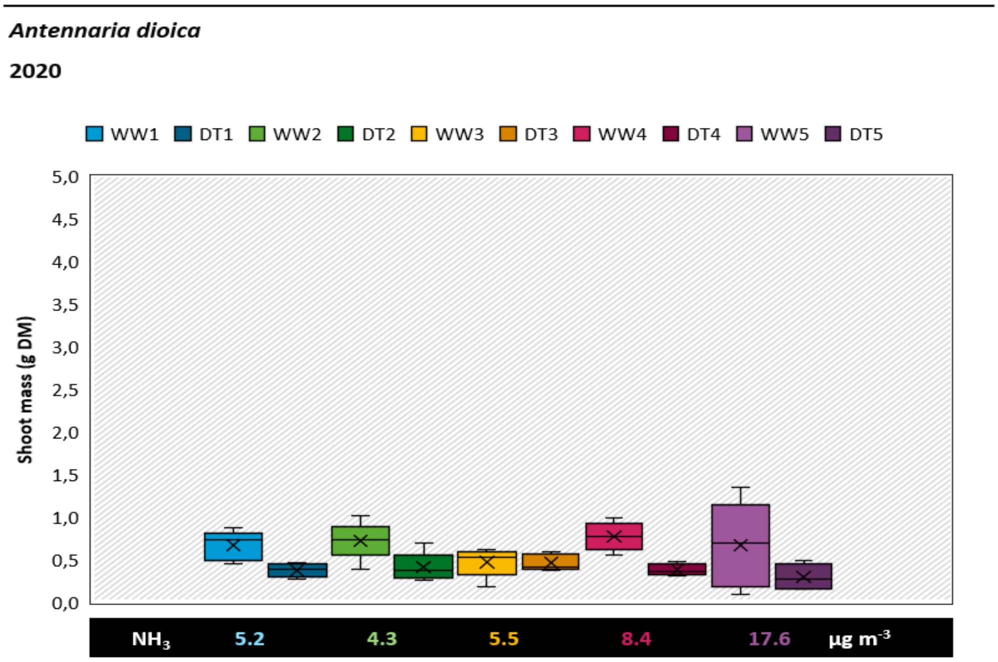
37c)

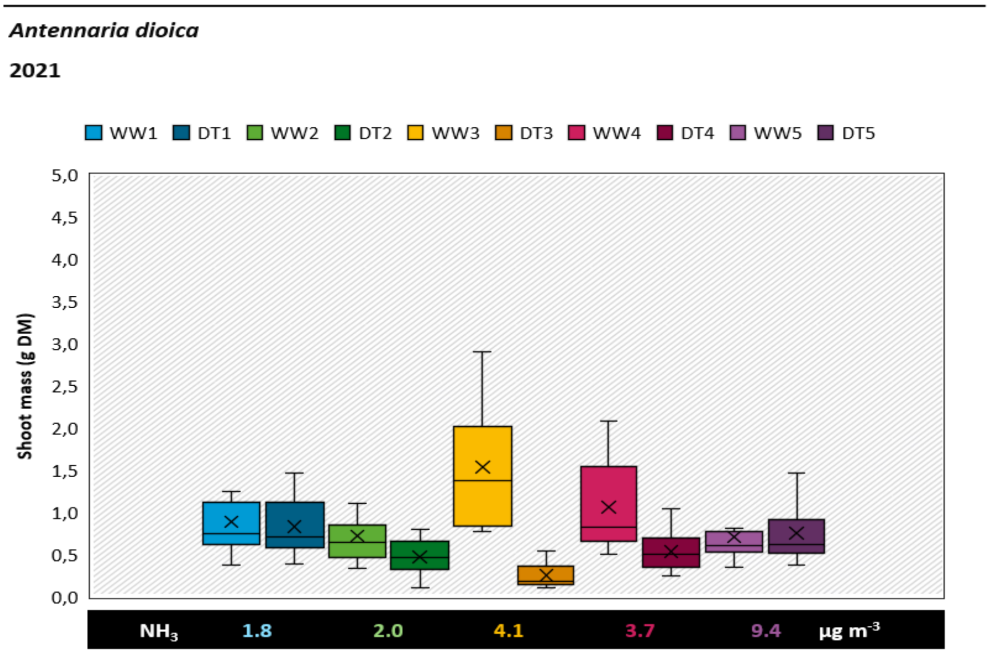


Source: Own illustration, University of Hohenheim.

Continued:

37d)

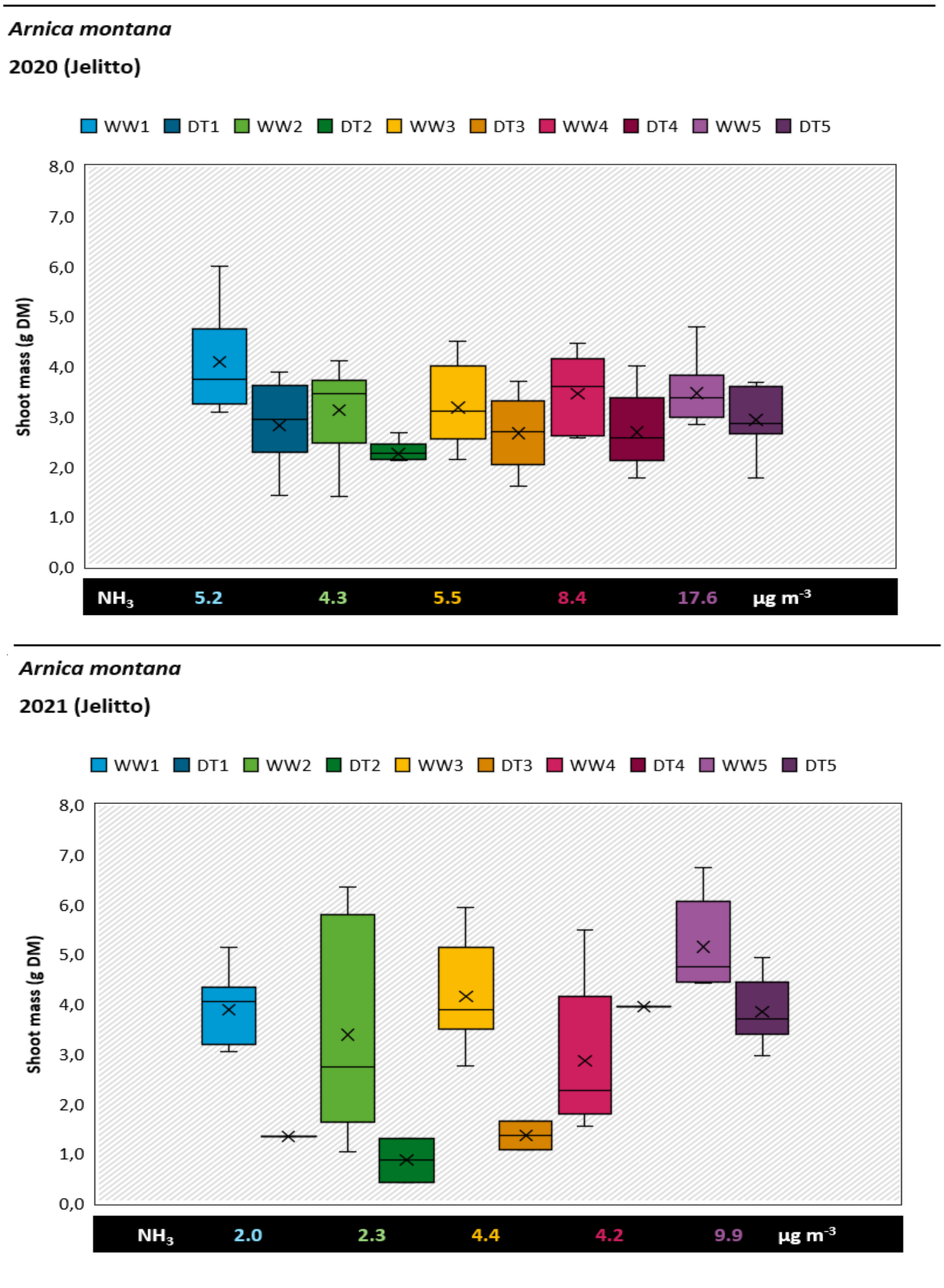




Source: Own illustration, University of Hohenheim.

Continued:

37e)



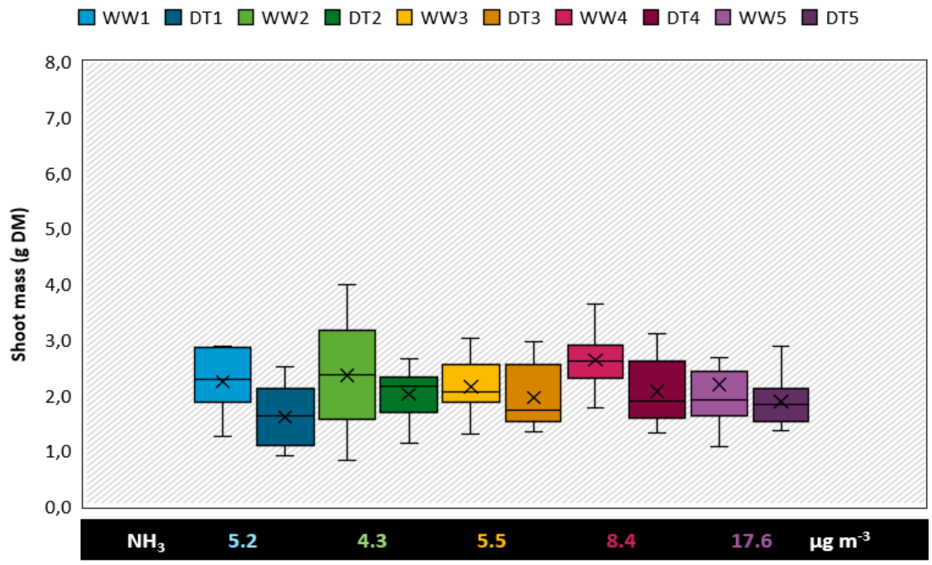
Source: Own illustration, University of Hohenheim.

Continued:

37f)

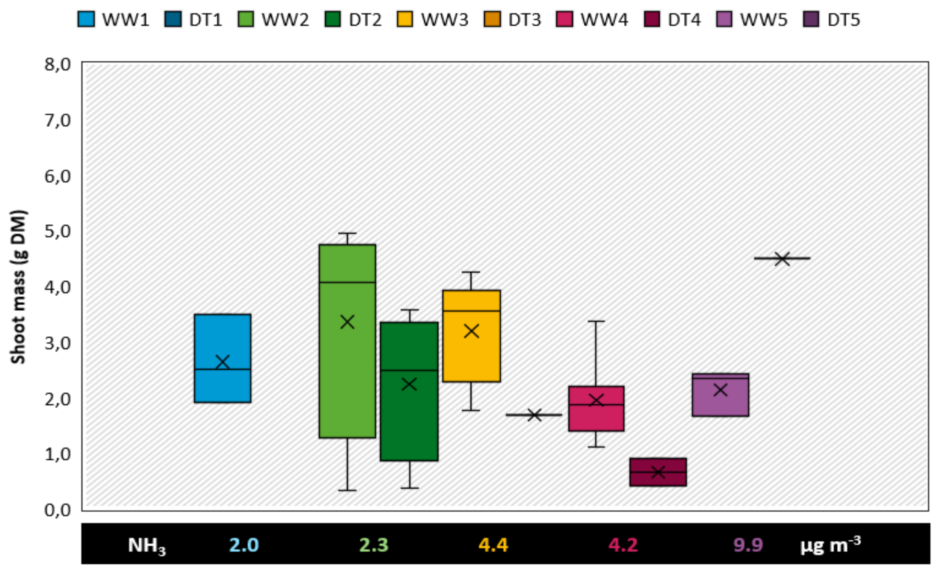
Arnica montana

2020 (Weiherberg)



Arnica montana

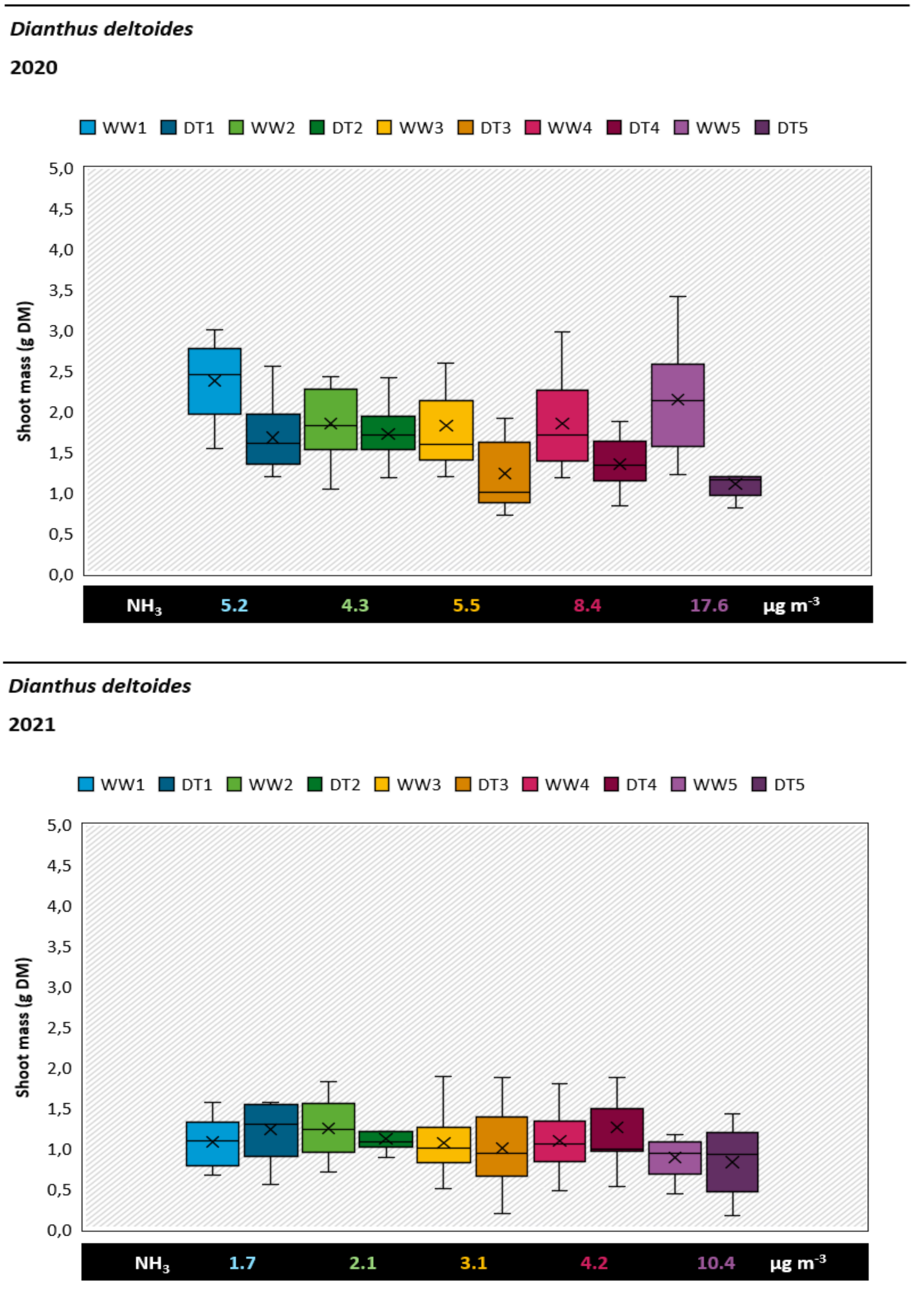
2021 (Weiherberg)



Source: Own illustration, University of Hohenheim.

Continued:

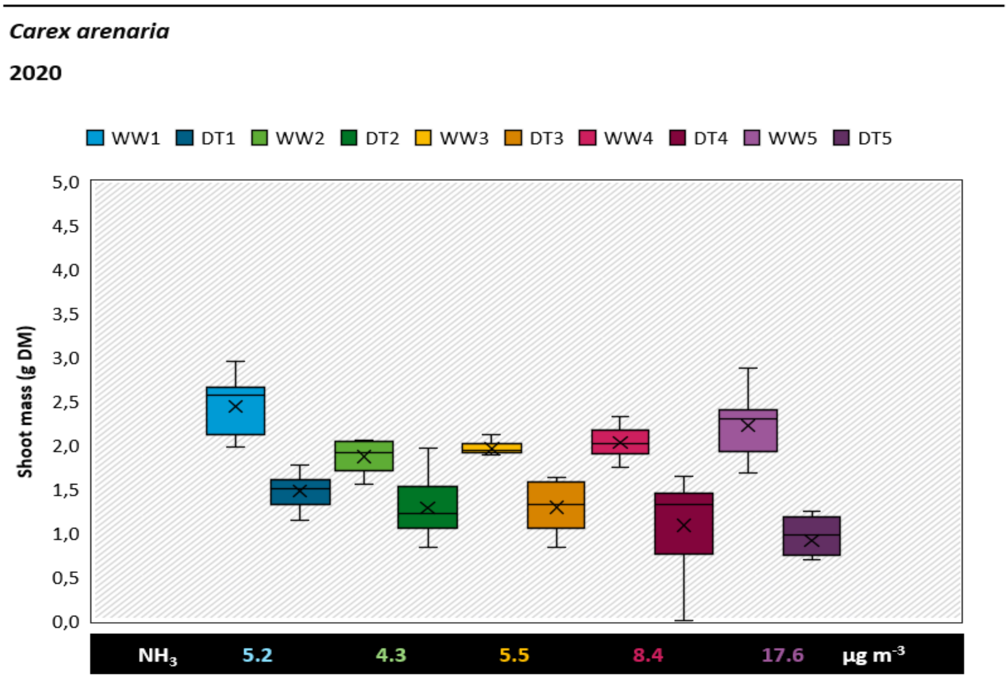
37g)

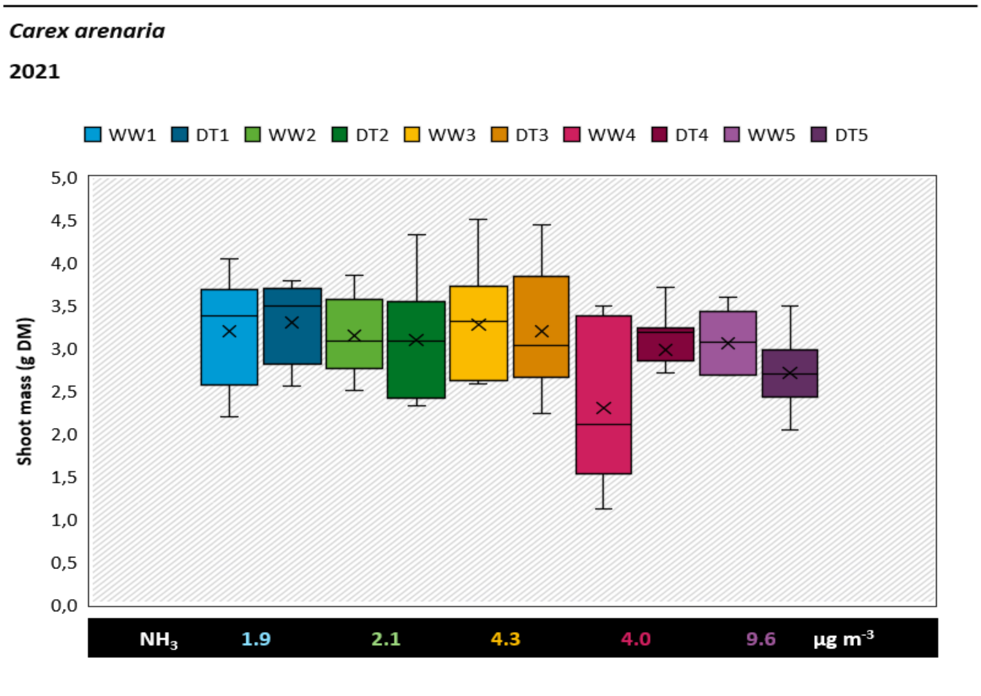


Source: Own illustration, University of Hohenheim.

Continued:

37h:





Source: Own illustration, University of Hohenheim.