



HAL
open science

Modelling the dynamic chemical interactions of atmospheric ammonia with leaf surface wetness in a managed grassland canopy

J. Burkhardt, Christophe Flechard, F. Gresens, M. Mattsson, P.A.C. Jongejan, J.W. Erisman, T. Weidinger, R Meszaros, E. Nemitz, M.A. Sutton

► To cite this version:

J. Burkhardt, Christophe Flechard, F. Gresens, M. Mattsson, P.A.C. Jongejan, et al.. Modelling the dynamic chemical interactions of atmospheric ammonia with leaf surface wetness in a managed grassland canopy. *Biogeosciences*, 2009, 6 (1), pp.67-84. 10.5194/bg-6-67-2009 . hal-01460922

HAL Id: hal-01460922

<https://hal.science/hal-01460922>

Submitted on 30 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Contribution of different grass species to plant-atmosphere ammonia exchange in intensively managed grassland

M. Mattsson^{1,*}, B. Herrmann², S. Jones^{2,3}, A. Nefitel², M. A. Sutton³, and J. K. Schjoerring¹

¹Plant and Soil Science Laboratory, University of Copenhagen, Faculty of Life Sciences, Thorvaldsensvej 40, 1871 Frederiksberg C, Copenhagen, Denmark

²Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, 8046 Zürich, Switzerland

³Natural Environmental Research Council, Centre for Ecology and Hydrology, Edinburgh Research Station, Pentlands EH26 0QB, Midlothian Scotland

* now at: Section for Economy and Technology, Halmstad University, Halmstad, 30118 Sweden

Received: 5 May 2008 – Published in Biogeosciences Discuss.: 17 June 2008

Revised: 4 December 2008 – Accepted: 4 December 2008 – Published: 9 January 2009

Abstract. Species diversity in grasslands usually declines with increasing input of nitrogen from fertilizers or atmospheric deposition. Conversely, species diversity may also impact the build-up of soil and plant nitrogen pools. One important pool is $\text{NH}_3/\text{NH}_4^+$ which also can be exchanged between plant leaves and the atmosphere. Limited information is available on how plant-atmosphere ammonia exchange is related to species diversity in grasslands. We have here investigated grass species abundance and different foliar nitrogen pools in 4-year-old intensively managed grassland. Apoplastic pH and NH_4^+ concentrations of the 8 most abundant species (*Lolium perenne*, *Phleum pratense*, *Festuca pratensis*, *Lolium multiflorum*, *Poa pratensis*, *Dactylis glomerata*, *Holcus lanatus*, *Bromus mollis*) were used to calculate stomatal NH_3 compensation points. Apoplastic NH_4^+ concentrations differed considerably among the species, ranging from 13 to 117 μM , with highest values in *Festuca pratensis*. Also apoplastic pH values varied, from pH 6.0 in *Phleum pratense* to 6.9 in *Dactylis glomerata*. The observed differences in apoplastic NH_4^+ and pH resulted in a large span of predicted values for the stomatal NH_3 compensation point which ranged between 0.20 and 6.57 nmol mol^{-1} . Three species (*Lolium perenne*, *Festuca pratensis* and *Dactylis glomerata*) had sufficiently high NH_3 compensation point and abundance to contribute to the bi-directional NH_3 fluxes recorded over the whole field. The other 5 grass species had NH_3 compensation points considerably below the atmospheric NH_3 concentration and were thus not likely to con-

tribute to NH_3 emission but only to NH_3 uptake from the atmosphere. Evaluated across species, leaf bulk-tissue NH_4^+ concentrations correlated well ($r^2=0.902$) with stomatal NH_3 compensation points calculated on the basis of the apoplastic bioassay. This suggests that leaf tissue NH_4^+ concentrations combined with data for the frequency distribution of the corresponding species can be used for predicting the NH_3 exchange potential of a mixed grass sward.

1 Introduction

The diversity of species in grasslands depends on a range of management and environmental factors (Cleland et al., 2006; Klimek et al., 2007; Pywell et al., 2007). In the short term, the initial composition of the seed mixture sown determines the proportion of different species in grasslands (Bullock et al., 2007), while in the longer term other management factors such as cutting frequency (Pontes et al., 2007; Critchley et al., 2007), fertilization (Hill and Carey, 1997; Oelmann et al., 2007) and liming (Silvertown et al., 2006) become important. Species diversity is usually reduced with increasing amounts of N fertilization (Clark et al., 2007; Harpole et al., 2007). Moderate to high rates of fertilizer N stimulate the more productive grass species, such as *Lolium perenne* and *Dactylis glomerata* (Whitehead, 1995; Hill et al., 2005). Increasing levels of atmospheric nitrogen deposition can also contribute to loss of species diversity (Stevens et al., 2004, 2006). With increasing age of the sward, the proportion of perennial ryegrass and other cultivated species will decline, and the number of indigenous species will increase (Hopkins, 1986; Critchley et al., 2002).



Correspondence to: J. K. Schjoerring
(jks@life.ku.dk)

There is a reciprocal relationship between species diversity and nitrogen abundance in grasslands: On the one hand, enhanced N input reduces species richness, while on the other hand the composition of grassland swards may affect the size of different soil and plant nitrogen pools (Oelmann et al., 2007). Limited information is available on how plant-atmosphere exchange of ammonia in grasslands is related to species diversity.

Studies of different grass species in cuvette systems under controlled laboratory conditions have shown that they can differ in both the rate of NH₃ exchange and in how the exchange is influenced by N nutrition (Hanstein et al., 1999; Mattsson and Schjoerring, 2002). Under field conditions, micrometeorological methods (Milford et al., 2008) and dynamic cuvette measurements (David et al., 2008) are used to study the exchange of ammonia between grassland vegetation and the atmosphere. However, none of these two approaches are able to distinguish between contributions from different species in a mixed canopy. An additional problem with micrometeorological measurements over grassland is that it is not possible with existing technology to separate between NH₃ emission from the foliage and emission from litter or soil.

Measurements of apoplastic NH₄⁺ and H⁺ concentrations under laboratory conditions (Husted and Schjoerring, 1995, 1996; Mattsson et al., 1997, 1998; Hanstein et al., 1999; Hill et al., 2001; Mattsson and Schjoerring, 2002) or in the field (Husted et al., 2000; Herrmann et al., 2001; van Hove et al., 2002; Loubet et al., 2002) have been useful for estimating the ammonia exchange potential of different species growing under different conditions. In order to develop more simple bio-indicators than those based on extraction of apoplastic solution it is essential to get information on the relationship between the stomatal NH₃ compensation point and other plant N pools. However, there is a limit to how simple things can be made as the total N content of the leaf tissue seems to be an inadequate parameter for prediction of the potential NH₃ emission from rye grass leaves (van Hove et al., 2002).

The aim of the present study was to measure different N parameters of the 8 most abundant species growing in a grass field. Apoplastic solution was analysed and the results used to predict the stomatal NH₃ compensation point for the different species. In addition, the concentrations of leaf bulk-tissue NH₄⁺, total soluble N and total N were measured and their correlation with the stomatal NH₃ compensation point analysed.

2 Materials and methods

2.1 Description of the measurement site

The investigation took place as part of the GRAMINAE integrated experiment between 22 May and 15 June 2000 (Sutton et al., 2008). The field site was located near Braunschweig in

Lower Saxony, Germany. The field was 600×300 m in size and consisted of a mixed sward. The field had been grassland for 4 years, receiving typically 250–350 kg N ha⁻¹ yr⁻¹. Some extra *Lolium perenne* seeds (20 kg/ha) had been inter-sown 2 months before the experiment. Plant species cover was assessed by point contact sampling at 20 points which were identified by a random walk between subsequent sampling points using four direction options and distances between 1 and 10 m (Knapp, 1984).

2.2 Sampling of plant material

Fully developed green leaves of the 8 most abundant grass species (*Lolium perenne*, *Phleum pratense*, *Festuca pratensis*, *Lolium multiflorum*, *Poa pratensis*, *Dactylis glomerata*, *Holcus lanatus*, *Bromus mollis*) were collected around noon on the 24 and 25 May 2000 for apoplastic pH and NH₄⁺ measurements. The plant material was collected randomly in the field and immediately brought to an adjacent field laboratory. Some leaves were immediately used for extraction of apoplastic solution and the rest were frozen in liquid nitrogen and stored at -20°C for later determination of tissue NH₄⁺, NO₃⁻ and total soluble N. For analysis of total N concentration, plant material was dried at 60°C for 24 h.

2.3 Extraction of apoplastic solution

Apoplast liquid was extracted by means of vacuum infiltration according to Husted and Schjoerring (1995). Whole leaf laminae were infiltrated with 280 mM sorbitol solution at a pressure of 16 bar and under vacuum for 5 s. This procedure was repeated 5 times. After infiltration, solution on leaf surfaces was removed by use of paper towels, whereupon the leaves were packed into plastic bags and left for 20 min in daylight in order to allow the apoplastic NH₄⁺ and H⁺ concentrations to reach equilibrium after infiltration. Thereafter, the leaves were centrifuged for 10 min at 4°C and 800 g. Concentrations of NH₄⁺ in the extracted solution were determined by flow injection analysis (FIA) using *o*-phthalaldehyde (OPA) as reagent (Genfa et al., 1989). Apoplastic pH was measured with a Micro-Combination pH electrode (type 9810, Orion, Beverly, USA). In order to assess cytoplasmic contamination of the apoplastic solution, malate dehydrogenase (MDH; E.C. 1.1.1.38) activity was determined and compared with the activity measured in bulk leaf extracts (Husted and Schjoerring, 1995). MDH activity of the apoplastic solution relative to bulk leaf extract was generally <1%.

2.4 Calculation of stomatal NH₃ compensation points

The stomatal NH₃ compensation point (χ_{NH_3} , mol NH₃ mol⁻¹ air) at 25°C was calculated by use of Eq. (1) derived from Husted and Schjoerring (1996) taking into

account that $K_d \ll [H^+]_{\text{apoplast}}$ within the range of apoplastic pH values:

$${}^{25}\chi_{\text{NH}_3} = K_{H,25} \times K_{d,25} \times \Gamma = 10^{-11.01} \times \Gamma \quad (1)$$

Γ is the dimensionless ratio between the apoplastic NH₄⁺ and H⁺ concentrations, and K_H and K_d are thermodynamic constants of $10^{-1.76} \text{ atm l mol}^{-1}$ and $10^{-9.25} \text{ mol l}^{-1}$ at 25°C, respectively. Equation (1) literally calculates the pressure of NH₃ (unit: atm), which according to Dalton's law of partial pressures is equal to the mol fraction (or volume fraction) at a given atmospheric pressure.

The calculated χ_{NH_3} at 25°C (T_{ref}) was adjusted to the actual canopy temperature T_c by the following equation derived from Husted and Schjoerring (1996):

$$\ln(T_c \chi_{\text{NH}_3} / T_{\text{ref}} \chi_{\text{NH}_3}) = (\Delta H_{\text{dis}}^0 + \Delta H_{\text{vap}}^0) / R \times (1/T_{\text{ref}} - 1/T_c) = 34.868 - 10395.91/T_c \quad (2)$$

$T_c \chi_{\text{NH}_3}$ is the requested NH₃ compensation point at the actual canopy temperature T_c (K), ΔH_{dis}^0 the enthalpy of NH₄⁺ dissociation (52.21 kJ mol⁻¹), ΔH_{vap}^0 the enthalpy of vaporization (34.18 kJ mol⁻¹), and R the gas constant (0.00831 kJ K⁻¹ mol⁻¹). The canopy temperature, measured by attached sensors as well as infra-red detection, was 16.7 and 20.7°C around noon on 24 and 25 May, respectively. Following the ideal gas law, an NH₃ compensation point of 1 nmol mol⁻¹ air corresponds to 0.71 μg NH₃ m⁻³ at 20°C.

2.5 Analysis of bulk tissue NH₄⁺, NO₃⁻, pH, total N and C

Frozen leaf samples were homogenised in 10 mM formic acid in a cooled mortar with a little sand. The homogenate was centrifuged at 25 000 g (2°C) for 10 min and the supernatant was transferred to 500-μl 0.45 μm polysulphone centrifugation filters (Micro VectraSpin, Whatman Ltd., Maidstone, UK) and spun at 5000 g (2°C) for 5 min (Husted et al., 2000b). The filtered solution was used for analysis of NO₃⁻ and NH₄⁺ concentrations on a flow injection system (Quick Chem instrument, Lachat Instruments INC, Milwaukee, USA). For bulk tissue pH measurements, 0.2 g sample of leaf material was homogenized in 2 ml of deionized water in a cooled mortar with a little sand. The homogenate was centrifuged at 14 000 g (4°C) for 10 min and pH in the supernatant measured with a microelectrode (Metrohm, Herisau, Switzerland). Tissue extracts were also analysed for total soluble N concentration (so-called substrate N) using an ANCA-SL Elemental Analyser coupled to a 20-20 Tracer-mass Mass Spectrometer (SerCon Ltd., Crewe, UK). The same equipment was used for analysis of total N and C concentrations in oven dried plant material ground to a fine powder.

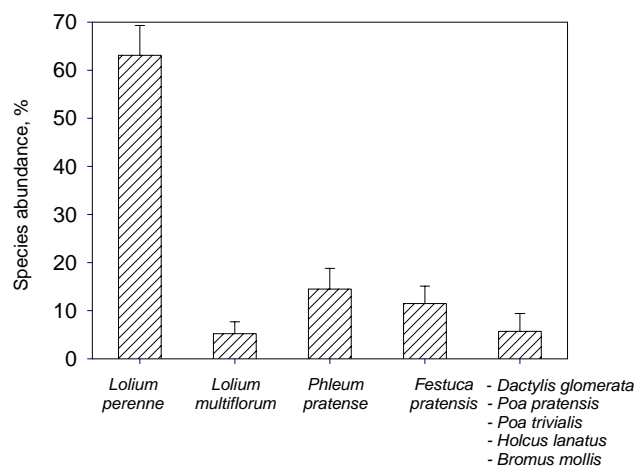


Fig. 1. Plant species abundance in a 4-year-old intensively managed grassland at Braunschweig, Germany. Data are means of 20 replicates \pm SE.

3 Results

3.1 Species diversity

The field site consisted of nine grass species, dominated by *Lolium perenne* and followed by *Phleum pratense*, *Festuca pratensis* and *Lolium multiflorum* (Fig. 1). Other grass species accounted for less than 10% of the total species composition. Relative to the composition of the seed mixture sown four years earlier (Sutton et al., 2008), *Lolium perenne* had increased in abundance from 29% to 63%, while *Festuca pratensis* and *Poa pratensis* had decreased from 33 to 11% and 12 to <5%, respectively. *Phleum pratense* had maintained approximately the same abundance as in the seed mixture. *Lolium multiflorum*, *Dactylis glomerata*, *Poa trivialis*, *Holcus lanatus* and *Bromus mollis* were not sown at all and must therefore be considered as invading species. *Festuca rubra* and *Trifolium repens* was also part of the seed mixture but had almost disappeared. All of the species measured were perennial grasses except the biennial *Lolium multiflorum* and the annual *Bromus mollis* (Table 1).

Using the standard set of indicator values for the Central European flora of Ellenberg (Ellenberg et al., 1991), the nitrogen preference of the different species in the experimental sward was compared. *Lolium perenne* had the highest N indicator value (Table 1) showing that this is a species found on rich fertile sites, while *Bromus mollis* had the lowest N value showing preference for nitrogen-poor sites (Table 1). The tendency of the species to dominate the sward was also compared on the basis of Ellenberg indicator values for dominance (Table 1). The observed richness of *Lolium perenne* (Fig. 1) was in agreement with the relatively high dominance indicator value for this species (Table 1). Also *Holcus lanatus* has a high dominance indicator value (Table 1) but was nevertheless only present in low abundance (Fig. 1) reflecting

Table 1. Life-cycle and Ellenberg indicator values on a scale from 1 to 9 for the 8 most abundant species growing in a 4-year-old intensively managed grassland at Braunschweig, Germany.

Species	Life-cycle	Dominance	N	R	F
<i>Lolium perenne</i>	perennial	6	7	7	5
<i>Dactylis glomerata</i>	perennial	5	6	X	5
<i>Phleum pratense</i>	perennial	5	6	7	5
<i>Holcus lanatus</i>	perennial	6	4	X	6
<i>Festuca pratensis</i>	perennial	5	6	X	6
<i>Bromus mollis (hordeaceus)</i>	annual	4	3	X	y
<i>Lolium multiflorum</i>	biennial	4	6	7	4
<i>Poa pratensis</i>	perennial	?	6	X	5

Dominance indicates tendency for dominating a sward (6: the species can dominate). Dominance is not known for *Poa pratensis*. N indicates preference for high or low N fertility (7: species found on highly fertile soil). R indicates preference for high or low soil pH (7: species found on neutral pH soils, never found on acid soils; X denotes high tolerance to both acidic and alkaline soils). F indicates preference for soil moisture conditions (5: moist-site indicator but not found on wet soils; y denotes high tolerance to both moist and dry conditions).

the fact that it was not initially sown in the field. The three species *Lolium perenne*, *Phleum pratense* and *Lolium multiflorum* that were most abundant in the sward (Fig. 1) had according to their Ellenberg indicator values preference for neutral soil pH and fairly moist soil (Table 1) matching the actual conditions at the field site (Sutton et al., 2008). Extra *Lolium perenne* seeds (20 kg/ha) had been intersown 2 months before the experiment and may have contributed to the high relative abundance of this species although it usually establishes rather slowly in an existing sward.

3.2 NH₃ exchange potential of individual grass species

In order to assess the potential for NH₃ exchange of each of the individual species, stomatal compensation points for NH₃ (χ_{NH_3}) were estimated on the basis of apoplastic NH₄⁺ concentrations and pH. Apoplastic NH₄⁺ concentrations differed considerably among the species, ranging from 13 to 117 μM , with highest values in *Festuca pratensis* (Fig. 2a). Also apoplastic pH values varied among the species, from pH 6.0 in *Phleum pratense* to 6.9 in *Dactylis glomerata* (Fig. 2b). The three species having the highest apoplastic NH₄⁺ concentrations also showed the highest apoplastic pH values. The observed differences in apoplastic NH₄⁺ and pH resulted in a large span of predicted values for χ_{NH_3} ranging between 0.20 and 6.57 nmol mol⁻¹ (Fig. 3). *Lolium perenne*, *Festuca pratensis* and *Dactylis glomerata* showed many-fold higher NH₃ compensation points compared to the rest of the species.

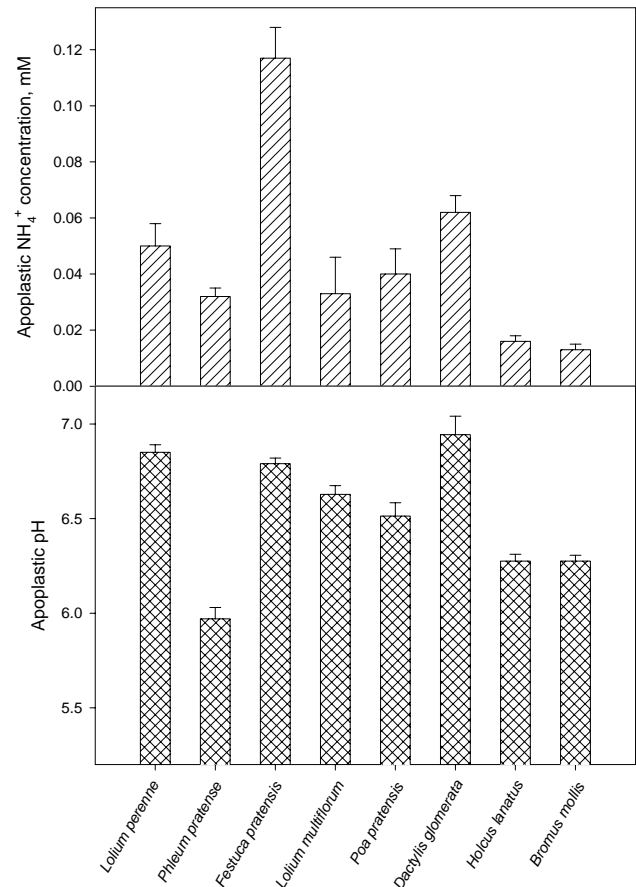


Fig. 2. A. Apoplastic NH₄⁺ concentrations and B. apoplastic pH values of different grass species growing in a 4-year-old intensively managed grassland at Braunschweig, Germany. Values are means \pm SE of 4 replicates.

3.3 Tissue level measurements

Bulk leaf tissue NH₄⁺ concentrations were about 25 times higher than the NH₄⁺ levels in the apoplastic solution (Fig. 4). The same three species having high apoplastic NH₄⁺ concentrations also showed highest bulk leaf tissue NH₄⁺ concentrations. Leaf tissue NO₃⁻ concentrations were in the same range as the NH₄⁺ concentrations with *Lolium perenne* having the highest value and *Phleum pratense* the lowest. The NH₄⁺ concentrations in bulk leaf tissue extracts of the 7 species (not enough leaf material was sampled of *Poa pratensis*) were well correlated with the apoplastic NH₄⁺ values although the best correlation was found between leaf tissue NH₄⁺ concentration and apoplastic Γ values (Fig. 5).

Substrate nitrogen, i.e. the total amount of soluble N measured in leaf extracts, also varied between the different species (Table 2). *Lolium perenne* showed more than twice the concentration of foliar substrate N compared to *Phleum pratense*. The linear relationship between tissue NH₄⁺ concentration and total soluble N concentration was

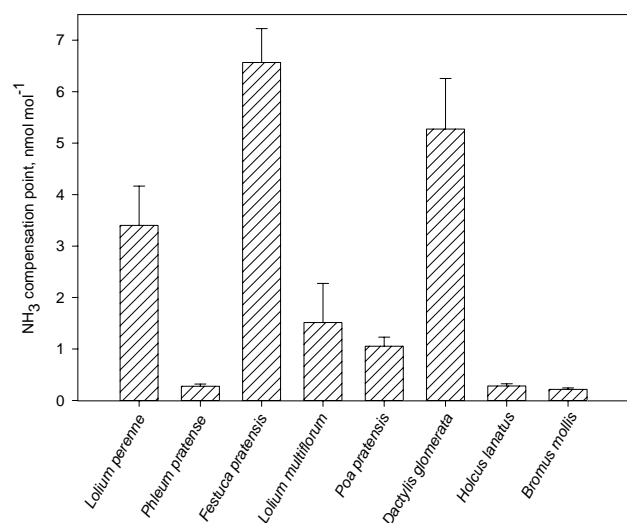


Fig. 3. Ammonia compensation points for 8 different grass species calculated on the basis of apoplastic NH₄⁺ and H⁺ concentrations analysed in a 4-year-old intensively managed grassland at Braunschweig, Germany. Values are means ± SE of 4 replicates.

weak ($R^2=0.287$, data not shown). Total N concentration on a dry weight basis ranged from 2.7% for *Phleum pratense* to 3.4% for *Bromus mollis*. The corresponding C:N ratios ranged between 17.3 and 13.2 (Table 2).

4 Discussion

The vacuum infiltration and centrifugation technique for extraction of foliar apoplastic solution enabled for the first time a comparison of the NH₃ exchange potential of as many as 8 grass species growing in a mixed sward. The sward was dominated by *Lolium perenne* (Fig. 1) but contained also a number of other species although 250–350 kg N ha⁻¹ year⁻¹ had been applied during the years 1996–2000. It has been shown that the proportion of cultivated species like *Lolium perenne* increases with the amount of fertilization but decline with age of the sward (Whitehead, 1995). Extraction of apoplastic solution was successfully applied to all plant species using a 280 mM (350 mOsm) sorbitol solution and an incubation time of 20 min between infiltration and extraction. Lohaus et al. (2001) found that osmolarity and incubation time had a relatively little influence on the composition of the apoplastic solution in 6 plant species.

Although growing in the same habitat, a large variability in apoplastic NH₄⁺ concentration occurred between the grass species (Fig. 2a). This may reflect their capability to adapt to the growth conditions of the field and the climatic conditions of the season (Table 1). *Holcus lanatus* and *Bromus mollis* having the lowest apoplastic NH₄⁺ concentrations are considered low-producing species under conditions of high fertilisation while *Lolium perenne* and *Dactylis glomerata* often

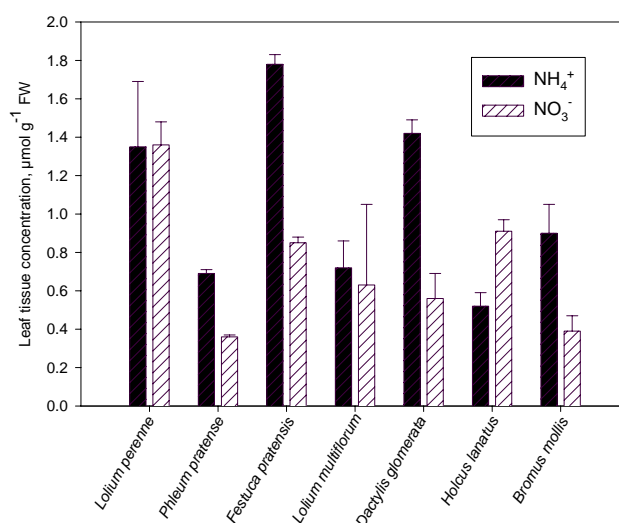


Fig. 4. Bulk leaf tissue NO₃⁻ and NH₄⁺ concentrations of different species growing in a 4-year-old intensively managed grassland at Braunschweig, Germany. Values are means ± SE of 4 replicates.

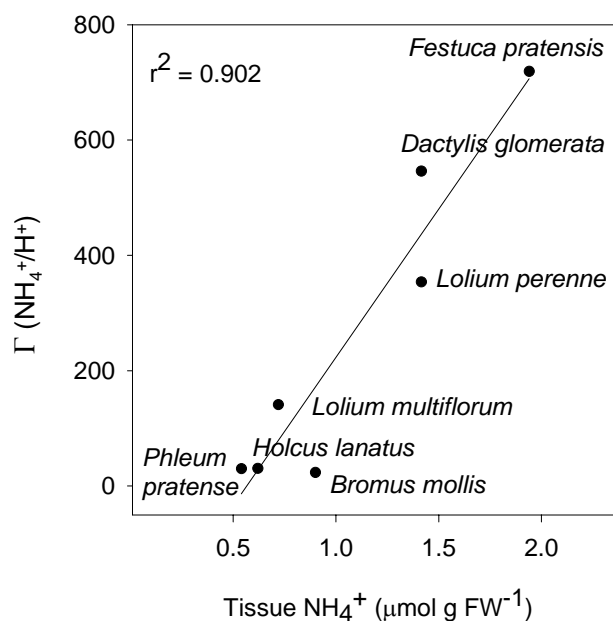


Fig. 5. Correlation between mean bulk leaf NH₄⁺ concentration and Γ (apoplastic NH₄⁺/H⁺) in leaves of different species growing in a 4-year-old intensively managed grassland at Braunschweig, Germany.

produce the highest yields and have the best responses to N fertilization. *Festuca pratensis* showed the highest apoplastic NH₄⁺ concentration of almost 0.12 mM and *Lolium perenne* and *Dactylis glomerata* had about half of that concentration, which is within the same range as apoplastic NH₄⁺ concentrations measured in the same two species in a grass/clover sward in Switzerland (Herrmann et al., 2001). Somewhat

Table 2. Total soluble N concentration, total N concentration and C:N ratio of fully developed green leaves of different grass species growing in a 4-year-old intensively managed grassland at Braunschweig, Germany. Means of 4 replicates \pm SE.

Species	Total soluble N (% of dry weight)	Total N (% of dry weight)	C:N ratio
<i>Lolium perenne</i>	0.84 \pm 0.12	3.2 \pm 0.2	14.3 \pm 0.7
<i>Phleum pratense</i>	0.37 \pm 0.03	2.7 \pm 0.03	17.3 \pm 0.2
<i>Festuca pratensis</i>	0.62 \pm 0.06	3.1 \pm 0.1	14.5 \pm 0.4
<i>Lolium multiflorum</i>	0.46 \pm 0.07	2.9 \pm 0.1	15.2 \pm 0.5
<i>Dactylis glomerata</i>	0.55 \pm 0.02	3.1 \pm 0.2	15.0 \pm 0.4
<i>Holcus lanatus</i>	0.45 \pm 0.04	3.0 \pm 0.1	14.6 \pm 0.4
<i>Bromus mollis</i>	0.55 \pm 0.08	3.4 \pm 0.1	13.2 \pm 0.3

higher values (0.2–0.9 mM) were measured in an intensively managed *Lolium perenne* grassland in the Netherlands (Loubet et al., 2002; van Hove et al., 2002). However, following application of nitrogen fertilizer, also the apoplastic NH₄⁺ concentrations of the grass mixture in the present field increased to around 0.9 mM (Mattsson et al., 2008). Apoplastic pH differed about one pH unit between the species with the lowest value (*Phleum pratense*; pH 6.0) and the highest value (*Dactylis glomerata*; pH 6.9). Over the period from January to November, van Hove et al. (2002) observed apoplastic pH values in *Lolium perenne* ranging between 5.9 and 6.5.

The three species *Lolium perenne*, *Festuca pratensis* and *Dactylis glomerata*, which had the highest apoplastic NH₄⁺ concentrations, also exhibited the highest apoplastic pH values (Fig. 2). The resulting NH₃ compensation points for these three species were 3.5, 6.5 and 5 nmol mol⁻¹, respectively. These compensation points are in agreement with results derived from laboratory cuvette studies, which showed NH₃ compensation points of 5.0 and 6.8 nmol mol⁻¹ for *Lolium perenne* and *Bromus erectus*, respectively, growing with a high concentration (3 mM) of NO₃⁻ in the nutrient solution (Mattsson and Schjoerring, 2002).

The atmospheric NH₃ concentration 1 m above ground during the period of apoplastic measurements (24 and 25 May 2000) was 3 to 4.5 nmol mol⁻¹ (corresponding to 2.1 to 3.2 μ g m⁻³). Since this value was lower than the predicted NH₃ compensation points, episodes of NH₃ emission would be expected, particularly since the total abundance of the three species with highest NH₃ compensation points was more than 70% (Fig. 1). Actually, NH₃ emission was measured around noon of 25 May, i.e. simultaneously with the sampling of the leaf material. Prior to cutting of the grass on 29 May, the NH₃ exchange pattern was dominated by small bi-directional fluxes (–64 to 42 ng NH₃ m⁻² s⁻¹; Milford et al., 2008). The predicted NH₃ compensation of the dominating species *Lolium perenne* (3.5 nmol mol⁻¹) was very close to the measured atmospheric NH₃ concentrations in the period. *Festuca pratensis* with the highest NH₃ compensation point of 6.5 nmol mol⁻¹ only accounted for 10% of the canopy in the experimental field and would thus not

be able to exert a major influence on the NH₃ exchange of the whole field. Furthermore, atmospheric NH₃ and/or NH₃ emitted from species with a high NH₃ compensation point may have been absorbed by some of the species with a low NH₃ compensation points such as *Phleum pratense* (Fig. 3) which had a fairly high abundance in the field (Fig. 1). Based on data from apoplastic measurements in intensively managed *Lolium perenne* grassland in the Netherlands, van Hove et al. (2002) estimated stomatal NH₃ compensation points varying between 0.7 and 6 nmol mol⁻¹ over the period from January to November. The gaseous NH₃ concentrations inside the grass leaves were, with a few exceptions, always smaller than the measured ambient NH₃ concentrations indicating that the grass canopy was unlikely to be a major source of NH₃ emission. In a study over non-fertilized managed grassland in The Netherlands, NH₃ emission fluxes were frequent (about 50% of the time) during a warm, dry summer period, while in a wet, cool autumn period deposition fluxes dominated (80% of the time) due to small canopy compensation points caused by low temperatures and a generally wet surface (Wichink Kruit et al., 2007).

Leaf tissue NH₄⁺ concentration ranged from 0.5 to 1.8 μ mol g⁻¹ FW (fresh weight) with the highest values obtained for the same three species that were in the top with respect to apoplastic NH₄⁺ concentration. NO₃⁻ concentrations were really high only in *Lolium perenne* (Fig. 4). *Phleum pratense* had low concentrations of both NH₄⁺ and NO₃⁻. Grass species are known to differ in their tendency to accumulate nitrate (Wilman and Wright, 1986) and particularly after fertilization when average leaf tissue NO₃⁻ concentrations increased to 40–50 μ mol g⁻¹ FW (Mattsson et al., 2008) the differences between species could have been more pronounced. Both soluble N and total N concentrations were also very low in *Phleum pratense* which led to an extremely high C:N ratio of 17.3 in the herbage compared to the other grass species (Table 2). On the other hand, *Bromus mollis*, which also showed low leaf tissue NH₄⁺ and NO₃⁻ concentrations, had the highest total N concentration in the leaves and therefore the lowest C:N ratio.

In the present investigation a clear linear relationship existed between leaf tissue NH₄⁺ concentration and apoplastic Γ values across species, but not between substrate N and Γ . Thus, tissue NH₄⁺ concentration proved to be a more promising indicator of NH₃ emission potential than substrate N. Similar correlations were also found for apoplastic and leaf tissue NH₄⁺ samples over a diurnal course (Herrmann et al., 2008) and over the entire experimental time course with different management events (Mattsson et al., 2008). The ratio between leaf tissue and apoplast NH₄⁺ concentration was for most species around 30 (Figs. 2 and 4). However, in *Holcus lanatus* and *Bromus mollis*, which contained a very low concentration of apoplastic NH₄⁺, this ratio was much higher. Prediction of NH₃ exchange potential from leaf tissue NH₄⁺ concentrations might therefore be problematic in some cases.

5 Conclusions

We conclude that grass species growing on the same field can differ greatly with respect to foliar N pools. Variations among grass species in apoplastic pH and NH₄⁺ concentrations may result in very different NH₃ compensation points. Species with high abundance and NH₃ compensation points around the level of atmospheric NH₃ concentrations may contribute to bi-directional NH₃ fluxes over the whole field, while species with considerably lower NH₃ compensation points only will contribute to NH₃ absorption from the atmosphere. In the present work, three species (*Lolium perenne*, *Festuca pratensis* and *Dactylis glomerata*) were likely to contribute to small NH₃ emissions (up to 42 ng NH₃ m⁻² s⁻¹) measured over the whole field during the day time. Leaf tissue NH₄⁺ concentrations were well correlated with the ratio between apoplastic NH₄⁺ and H⁺ concentrations suggesting that leaf tissue NH₄⁺ can be used for predicting the NH₃ exchange potential.

Acknowledgements. The authors gratefully acknowledge the support of many different funders and colleagues to this work. The measurements were conducted under the frame of the GRAMINAE project funded by the European Commission (ENV4-CT98-0722). The final analysis of results was conducted under the EC NitroEurope Integrated Project.

Edited by: F. X. Meixner

References

- Bullock, J. M., Pywell, R. F., and Walker, K. J.: Long-term enhancement of agricultural production by restoration of biodiversity, *J. Appl. Ecol.*, 44, 6–12, 2007.
- Clark, C. M., Cleland, E. E., Collins, S. L., Fargione, J. E., Gough, L., Gross, K. L., Pennings, S. C., Suding, K. N., and Grace, J. B.: Environmental and plant community determinants of species loss following nitrogen enrichment, *Ecol. Lett.*, 10, 596–607, 2007.
- Cleland, E. E., Chiariello, N. R., Loarie, S. R., Mooney, H. A., and Field, C. B.: Diverse responses of phenology to global changes in a grassland ecosystem, *Proc. Natl. Acad. Sci. USA*, 103, 13740–13744, 2006.
- Critchley, C. N. R., Chambers, B. J., Fowbert, J. A., Sanderson, R. A., Bhogal, A., and Rose, S. C.: Association between lowland grassland plant communities and soil properties, *Biol. Conserv.*, 105, 199–215, 2002.
- Critchley, C. N. R., Fowbert, J. A., and Wright, B.: Dynamics of species-rich upland hay meadows over 15 years and their relation with agricultural management practices, *Appl. Veg. Sci.*, 10, 307–314, 2007.
- David, M., Loubet, B., Cellier, P., Mattsson, M., Schjoerring, J. K., Nemitz, E., Roche, R., Riedo, M., and Sutton, M. A.: Ammonia sources and sinks in an intensively managed grassland using dynamic chambers, *Biogeosciences Discuss.*, accepted, 2008.
- Ellenberg, H., Weber H. E., Düll, R., Wirth, V., Werner, W., and Paulissen, D.: Indicator values of plants in Central Europe, *Scripta Geobotanica*, 18, 1, 1–248, 1991.
- Genfa, Z., Dasgupta, P. K., and Dong, S.: Measurement of atmospheric ammonia, *Environ. Sci. Technol.*, 23, 1467–1474, 1989.
- Hanstein, S., Mattsson, M., Jaeger, H.-J., and Schjoerring, J. K.: Uptake and utilization of atmospheric ammonia in three native *Poaceae* species: Leaf conductances, composition of apoplastic solution and interactions with nitrogen supply, *New Phytol.*, 141, 71–83, 1999.
- Harpole, W. S., Potts, D. L., and Suding, K. N.: Ecosystem responses to water and nitrogen amendment in a California grassland, *Global Change Biol.*, 13, 2341–2348, 2007.
- Herrmann, B., Jones, S. K., Fuhrer, J., Feller, U., and Neftel, A.: N budget and NH₃ exchange of a grass/clover crop at two levels of N application, *Plant Soil*, 235, 243–252, 2001.
- Hill, J. O., Simpson, R. J., Wood, J. T., Moore, A. D., and Chapman, D. F.: The phosphorus and nitrogen requirements of temperate pasture species and their influence on grassland botanical composition, *Aust. J. Agric. Res.*, 56, 1027–1039, 2005.
- Hill, M. O. and Carey, P. D.: Prediction of yield in the Rothamsted Park Grass Experiment by Ellenberg indicator values, *J. Veg. Sci.*, 8, 579–586, 1997.
- Hill, P. W., Raven, J. A., Loubet, B., Fowler, D., and Sutton, M. A.: Comparison of gas exchange and bioassay determinations of the ammonia compensation point in *Luzula sylvatica* (Huds.) Gaud., *Plant Physiol.*, 125, 476–487, 2001.
- Hopkins, A.: Botanical composition of permanent grassland in England and Wales in relation to soil, environment and management factors, *Grass Forage Sci.*, 41, 237–246, 1986.
- Husted, S. and Schjoerring, J. K.: Apoplastic pH and ammonium concentration in leaves of *Brassica napus* L., *Plant Physiol.*, 109, 1453–1460, 1995.
- Husted, S. and Schjoerring, J. K.: Ammonia flux between oilseed rape plants and the atmosphere in response to changes in leaf temperature, light intensity, and air humidity, *Plant Physiol.*, 112, 67–74, 1996.
- Husted, S., Schjoerring, J. K., Nielsen, K. H., Nemitz, E., and Sutton, M. A.: Stomatal compensation points for ammonia in oilseed rape plants under field conditions, *Agr. Forest Meteorol.*, 105, 371–383, 2000a.
- Husted, S., Hebborn, C. A., Mattsson, M., and Schjoerring, J. K.: Determination of ammonium, low molecular weight amines and

- amides in plant tissue, *Physiol. Plant.*, 109, 167–179, 2000b.
- Klimek, S., Kemmermann, A. R. G., Hofmann, M., and Isselstein, J.: Plant species richness and composition in managed grasslands: The relative importance of field management and environmental factors, *Biol. Conserv.*, 134, 559–570, 2007.
- Knapp, R. (Ed.): Sampling methods in taxon analysis in vegetation science, *Handbook of Vegetation Science 1. Part 4*, Junk, Hague, The Netherlands, 1984.
- Lohaus, G., Pennewiss, K., Sattelmacher, B., Hussmann, M., and Muehling, K. H.: Is the infiltration-centrifugation technique appropriate for isolation of apoplastic fluid? A critical evaluation with different plant species, *Physiol. Plant.*, 111, 457–465, 2001.
- Loubet, B., Milford, C., Hill, P. W., Tang, Y. S., Cellier, P., and Sutton, M. S.: Seasonal variability of apoplastic NH₄⁺ and pH in an intensively managed grassland, *Plant Soil*, 238, 97–110, 2002.
- Mattsson, M., Häusler, R. E., Leegood, R. C., Lea, P., and Schjoerring, J. K.: Leaf-atmosphere ammonia exchange in barley mutants with reduced activities of glutamine synthetase, *Plant Physiol.*, 114, 1307–1312, 1997.
- Mattsson, M., Husted, S., and Schjoerring, J. K.: Influence of nitrogen nutrition and metabolism on ammonia volatilization in plants, *Nutr. Cycl. Agroecosys.*, 51, 35–40, 1998.
- Mattsson, M. and Schjoerring, J. K.: Dynamic and steady state responses of inorganic nitrogen pools and NH₃ exchange in leaves of *Lolium perenne* and *Bromus erectus* to changes in root N supply, *Plant Physiol.*, 128, 742–750, 2002.
- Mattsson, M., Herrmann, B., David, M., Loubet, B., Riedo, M., Theobald, M. R., Sutton, M. A., Bruhn, D., Neftel, A., and Schjoerring, J. K.: Temporal variability in bioassays of ammonia exchange potential in relation to plant and soil nitrogen parameters in intensively managed grassland, *Biogeosciences Discuss.*, 5, 2749–2772, 2008, <http://www.biogeosciences-discuss.net/5/2749/2008/>.
- Milford, C., Theobald, M. R., Nemitz, E., Hargreaves, K. J., Horvath, L., Raso, J., Dämmgen, U., Neftel, A., Jones, S. K., Hensen, A., Loubet, B., Cellier, P., and Sutton, M. A.: Ammonia fluxes in relation to cutting and fertilization of an intensively managed grassland derived from an inter-comparison of gradient measurements, *Biogeosciences Discuss.*, 5, 4699–4744, 2008, <http://www.biogeosciences-discuss.net/5/4699/2008/>.
- Mountford, J. O., Lakhani, K. H., and Kirkham, F. W.: Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor, *J. Appl. Ecol.*, 30, 321–332, 1993.
- Nielsen, K. H. and Schjoerring, J. K.: Regulation of apoplastic NH₄⁺ concentration in leaves of oilseed rape, *Plant Physiol.*, 118, 1361–1368, 1998.
- Oelmann, Y., Wilcke, W., Temperton, V. M., Buchmann, N., Roscher, C., Schumacher, J., Schulze, E. D., and Weisser, W. W.: Soil and plant nitrogen pools as related to plant diversity in an experimental grassland, *Soil Sci. Soc. Am. J.*, 71, 720–729, 2007.
- Pontes, L. S., Carrere, P., Andueza, D., Louault, F., and Soussana, J. F.: Seasonal productivity and nutritive value of temperate grasses found in semi-natural pastures in Europe: responses to cutting frequency and N supply, *Grass Forage Sci.*, 62, 485–496, 2007.
- Pywell, R. F., Bullock, J. M., Tallowin, J. B., Walker, K. J., Warman, E. A., and Masters, G.: Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints, *J. Appl. Ecol.*, 44, 81–94, 2007.
- Schjoerring, J. K. and Mattsson, M.: Quantification of ammonia exchange between agricultural cropland and the atmosphere: Measurements over two complete growth cycles of oilseed rape, wheat, barley and pea, *Plant Soil*, 228, 105–115, 2001.
- Silvertown, J.: The dynamics of a grassland ecosystem: botanical equilibrium in the Park grass experiment, *J. Appl. Ecol.*, 17, 491–504, 1980.
- Silvertown, J., Poulton, P., Johnston, E., Edwards, G., Heard, M., and Biss, P. M.: The Park Grass Experiment 1856–2006: Its contribution to ecology, *J. Ecol.*, 94, 801–814, 2006.
- Stevens, C. J., Dise, N. B., Gowing, D. J. G., and Mountford, J. O.: Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls, *Global Change Biol.*, 12, 1823–1833, 2006.
- Stevens, C. J., Dise, N. B., Mountford, J. O., and Gowing, D. J.: Impact of nitrogen deposition on the species richness of grasslands, *Science*, 303, 1876–1879, 2004.
- Sutton, M. A., Nemitz, E., Theobald, M. R., Milford, C., Dorsey, J. R., Gallagher, M. W., Hensen, A., Jongejan, P. A. C., Erisman, J. W., Mattsson, M. E., Schjoerring, J. K., Cellier, P., Loubet, B., Roche, R., Neftel, A., Hermann, B., Jones, S., Lehman, B. E., Horvath, L., Weidinger, T., Rajkai, K., Burkhardt, J., Löpmeier, F. J., and Daemmgen, U.: Dynamics of ammonia exchange with cut grassland: strategy and implementation of the GRAMINAE Integrated Experiment, *Biogeosciences Discuss.*, 5, 3347–3407, 2008, <http://www.biogeosciences-discuss.net/5/3347/2008/>.
- van Hove, L. W. A., Heeres, P., and Bossen, M. E.: The annual variation in stomatal ammonia compensation point of rye grass (*Lolium perenne* L.) leaves in an intensively managed grassland, *Atmos. Environ.*, 36, 2965–2977, 2002.
- Whitehead, D. C.: Grasses: Uptake of nitrogen and effects on morphology and physiology, in: *Grassland nitrogen*, CAB International, edited by: Whitehead, D. C., UK, 16–34, 1995.
- Wichink Kruit, R. J., van Pul, W. A. J., Otjes, R. P., Hofschreuder, P., Jacobs, A. F. G., and Holtslag, A. A. M.: Ammonia fluxes and derived canopy compensation points over non-fertilized agricultural grassland in The Netherlands using the new gradient ammonia-high accuracy-monitor (GRAHAM), *Atmos. Environ.*, 41, 1275–1287, 2007.
- Wilman, D. and Wright, P. T.: The effect of interval between harvests and nitrogen application on the concentration of nitrate-nitrogen in the total herbage, green leaf and “stem” of grasses, *J. Agric. Sci., Cambridge*, 106, 467–475, 1986.