Biogeosciences Discuss., 5, 2583–2605, 2008
www.biogeosciences-discuss.net/5/2583/2008/
© Author(s) 2008. This work is distributed under
the Creative Commons Attribution 3.0 License.

Biogeosciences Discussions

Biogeosciences Discussions is the access reviewed discussion forum of *Biogeosciences*

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

M. Mattsson[1,*], B. Herrmann[2], S. Jones[2,3], A. Neftel[2], M. A. Sutton[3], and J. K. Schjoerring[1]

[1]Plant and Soil Science Laboratory, University of Copenhagen, Faculty of Life Sciences, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark
[2]Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH -8046 Zürich, Switzerland
[3]Natural Environmental Research Council, Centre for Ecology and Hydrology, Edinburgh Research Station, Penicuik EH26 0QB, Midlothian Scotland
[*]now at: Section for Economy and Technology, Halmstad University, Halmstad, S-30118 Sweden

Received: 5 May 2008 – Accepted: 19 May 2008 – Published: 17 June 2008

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

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Species diversity in grasslands usually declines with increasing input of nitrogen from fertilizers or atmospheric nitrogen deposition. Conversely, species diversity may also impact the build-up of soil nitrogen pools. 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 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 from 0.20 to 6.57 nmol mol^{-1}. Three species (*Lolium perenne, Festuca pratensis* and *Dactylis glomerata*) had sufficiently high NH_3 compensation points and abundance to contribute to the NH_3 emission of the whole field. At the same time, other grass species such as *Phleum pratense* and *Lolium multiflorum* had NH_3 compensation points below the atmospheric NH_3 concentration and could thus contribute 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

BGD

5, 2583–2605, 2008

short term, the initial composition of the seed mixture sown determines the proportion of different species 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 usually 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).

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. Micrometeorological measurements of NH_3 volatilisation over a grass sward can not distinguish between contributions from different species. Another problem with micro-meteorological measurements over grassland is that it is not possible with existing technology to separate between NH_3 emission from the foliage and emission from litter or soil. Most studies of NH_3 volatilisation from different grass species have been carried out in cuvette systems under controlled laboratory conditions. Such studies have shown that grass species can differ in both rate of NH_3 exchange and in how the exchange is influenced by N nutrition (Hanstein et al., 1999; Mattsson and Schjoerring, 2002). As an alternative to gas exchange measurements, the NH_3 exchange potential by plants may be estimated from apoplastic NH_4^+ and H^+ concentrations as demonstrated under both laboratory conditions (Husted and Schjoerring, 1995, 1996; Mattsson et al., 1998; Hanstein et al., 1999; Hill et al., 2001; Mattsson and Schjoer-

BGD
5, 2583–2605, 2008

ring, 2002) and in the field (Husted et al., 2000; Herrmann et al., 2001; van Hove et al., 2002; Loubet et al., 2002). In order to develop more simple bio-indicators for the potential NH_3 emission than those based on extraction of apoplastic solution it is essential to get information on the relationship between the stomatal NH_3 compensation point and other plant N pools. The total N content of the leaf tissue has been shown to be inadequate parameter for prediction of the potential NH_3 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 NH_3 compensation point for the different species. In addition, the concentrations of leaf bulk-tissue NH_4^+, total soluble N and total N were measured and their correlation with the stomatal NH_3 compensation point analysed.

2 Materials and methods

2.1 Description of the measurement site

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}. 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).

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 for apoplastic pH and NH_4^+ measurements. The plant material was collected randomly in the field and immediately

CC BY

brought to an adjacent field laboratory. Some leaves some 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 NH_4^+, NO_3^- 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 laminas 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, leaves were carefully blotted dry, packed into plastic bags and left to equilibrate for 20 min in day light in order to reach complete homeostasis of the apoplastic NH_4^+ concentration. Thereafter the leaves were centrifuged for 10 min at 4°C and 800 g. Concentrations of NH_4^+ 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 apoplasts, malate dehydrogenase (E.C. 1.1.1.38) activity was determined and compared with the activity measured in bulk leaf extracts (Husted and Schjoerring, 1995).

2.4 Calculation of stomatal NH_3 compensation points

The stomatal NH_3 compensation point (χ_{NH_3}) at 25°C, ${}^{25}\chi_{NH_3}$, was calculated as:

$$ {}^{25}\chi_{NH_3} = K_{H.25} \times K_{d.25} \times \Gamma = 10^{-11.01} \times \Gamma \quad (1) $$

Where Γ is the ratio between the apoplastic NH_4^+ and H^+ concentrations, and K_H and K_d are thermodynamic constants of $10^{-9.25}$ and $10^{-1.76}$ at 25°C, respectively (Husted and Schjoerring, 1996). The calculated χ_{NH_3} at 25°C was adjusted to the actual canopy temperature $t2$ in °C by the following equation derived from Husted and

CC BY

Schjoerring (1996):

$$\ln({}^{t2}\chi_{NH_3}/{}^{25}\chi_{NH_3}) = (\Delta H^{\circ}_{dis} + \Delta H^{\circ}_{vap})/R \times (1/298.15 - 1/T_2) = 34.868 - 10395.91/(273.15 + t_2) \quad (2)$$

${}^{t2}\chi_{NH_3}$ is the requested NH_3 compensation point at the actual temperature t_2 (°C), ΔH°_{dis} the enthalpy of NH_4^+ dissociation (52.21 kJ mol^{-1}), ΔH°_{vap} the enthalpy of vaporization (34.18 kJ mol^{-1}), and R the gas constant (8.31 J K^{-1} mol).

2.5 Determination of bulk tissue NH_4^+ and NO_3^- and total N concentration

Frozen leaf samples were homogenised in 10 mM formic acid in a cooled mortar with a little sand. The homogenate was centrifuged at 25000 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 NO_3^- and NH_4^+ concentrations on a flow injection system (Quick Chem instrument, Lachat Istruments INC, Milwaukee, USA). 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 Tracermass Mass Spectrometer (Europa Scientific 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.

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, *Lolium perenne* had

increased in abundance from 29% to 63%, while *Festuca pratensis* and *Poa pratensis* had decreased in abundance from 33 to 11% and 12 to <5%, respectively (Sutton et al., 2008a[1]). *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* had also been 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 almost infertile 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 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 preferences for neutral soil pH and fairly moist soil (Table 1) matching the actual conditions at the field site (Sutton et al., 2008a[1]).

[1]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., Herrmann, B., Jones, S. K., Lehman, B. E., Horvath, L., Weidinger, T., Rajkai, K., Burkhardt, J., Lpmeier, F. J., Dmmgen, U.: Dynamics of ammonia exchange with cut grassland: Strategy and implementation of the GRAMINAE Integrated Experiment, Biogeosciences Discuss., submitted, 2008a.

BGD
5, 2583–2605, 2008

3.2 NH_3 exchange potential of individual grass species

In order to assess the potential for NH_3 exchange of each of the individual species, stomatal compensation points for NH_3 (χ_{NH_3}) were estimated on the basis of apoplastic NH_4^+ concentrations and pH. Apoplastic NH_4^+ 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 NH_4^+ concentrations also showed the highest apoplastic pH values. The observed differences in apoplastic NH_4^+ and pH variations resulted in a large span of predicted values for χ_{NH3}ranging between 0.20 and 6.57 nmol mol^{-1}(Fig. 3). *Lolium perenne, Festuca pratensis* and *Dactylis glomerata* showed many-fold higher NH_3 compensation points compared to the rest of the species.

3.3 Tissue level measurements

Bulk leaf tissue NH_4^+ concentrations were about 30 times higher than the NH_4^+ levels in the apoplastic solution (Fig. 4). The same three species having high apoplastic NH_4^+concentrations also showed highest bulk leaf tissue NH_4^+ concentrations. Leaf tissue NO_3^- concentrations were in the same range as the NH_4^+ concentrations with *Lolium perenne* having the highest value and *Phleum pratense* the lowest. The NH_4^+ concentrations in bulk leaf tissue extracts of the 7 species (not enough leaf material was sampled of *Poa pratensis)* were usually well correlated with the apoplastic NH_4^+ values although the best correlation was found between leaf tissue NH_4^+ concentration and Γ (Fig. 5).

Substrate nitrogen in leaf tissue, 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 soluble N in leaves compared to *Phleum pratense*. The linear relationship between tissue NH_4^+ concentration and total soluble N concen-

BGD
5, 2583–2605, 2008

tration was weak (R^2= 0.287, data not shown). Total N concentration on a dry weight basis ranged between 2.7% for *Phleum pratense* and 3.4% for *Bromus mollis* and 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 NH_3 exchange potential of as many as 8 grass species growing in a mixed sward. The sward was 4 years old and was therefore not completely dominated by *Lolium perenne* (Fig. 1) even though 250-350 kg N ha^{-1} $year^1$ had been applied during the years 1996–2000 and some extra *Lolium perenne* seeds (20 kg ha^{-1}) had been sown 2 months before the experiment. It has been shown that the proportion of cultivated species like *Lolium perenne* increase with 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. Cytoplasmic contamination expressed as MDH activity of the apoplast relative to the bulk leaf extract was generally below 1% (data not shown). An incubation time of 20 min between infiltration and extraction was used for all the species in order to ensure full equilibration. That the grass species should differ in this respect was not very likely since a previous experiment had shown that both *Lolium perenne* and *Bromus erectus* needed this time to adjust the NH_4^+ and H^+ concentrations after infiltration (unpublished results). Lohaus et al. (2001) also 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 NH_4^+ 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 NH_4^+

CC BY

concentrations are considered low producing species under conditions of high fertilisation while *Lolium perenne* and *Dactylis glomerata* often produce the highest yields and the best responses to N fertilization. *Festuca pratensis* showed the highest apoplastic NH_4^+ 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 NH_4^+ concentrations measured in the same two species in a grass/clover sward in Switzerland (Herrmann et al., 2001), while somewhat higher values (0.2–0.9 mM) were measure 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 NH_4^+ concentrations of the grass mixture in the present field increased to around 0.9 mM (Mattsson et al., 2008[2]). 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 NH_4^+ concentrations, also exhibited the highest apoplastic pH values (Fig. 2). The resulting NH_3 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 NH_3 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 NO_3^- in the nutrient solution (Mattsson and Schjoerring, 2002).

The atmospheric NH_3 concentration 1 m above ground during the period of apoplas-

[2]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 emission potential in relation to plant and soil N parameters in intensively managed grassland, Biogeosciences Discuss., submitted, 2008.

CC BY

tic measurements was 3 to 4.5 nmol mol^{-1} (Milford et al., 2008[3]). Since this value was lower than the predicted NH_3 compensation points, episodes of NH_3 emission would be expected, particularly since the total abundance of the three species with highest NH_3 compensation points was more than 70% (Fig. 1). However, the measured NH_3 fluxes indicated mostly small NH_3 deposition during the period (Milford et al., 2008[3]). This may be due to the fact that the predicted NH_3 compensation of the dominating species *Lolium perenne* (3.5 nmol mol^{-1}) actually was very close to the measured atmospheric NH_3 concentrations and that *Festuca pratensis* with the highest NH_3 compensation point of 6.5 nmol mol^{-1} only accounted for 10% of the canopy in the experimental field. Furthermore, atmospheric NH_3 and/or NH_3 emitted from species with a high NH_3 compensation point may have been absorbed by some of the species with a low NH_3 compensation points such as *Phleum pratense* (Fig. 3) which has 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 NH_3 compensation points varying between 0.7 and 6 nmol mol^{-1} over the period from January to November. The gaseous NH_3 concentrations inside the grass leaves were, with a few exceptions, always smaller than the measured ambient NH_3 concentrations indicating that the grass canopy was unlikely to be a major source of NH_3 emission. In a study over non-fertilized managed grassland in The Netherlands, NH_3 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 NH_4^+ concentration ranged from 0.5 to 1.8 μmol g^{-1} FW with the highest values obtained for the same three species that were in the top with respect to

[3]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., 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., submitted, 2008.

CC BY

apoplastic NH_4 concentration. NO_3^- concentrations were really high only in *Lolium perenne* (Fig. 4). *Phleum pratense* had low concentrations of both NH_4^+ and NO_3^-. Grass species are known to differ in their tendency to accumulate nitrate (Wilman and Wright, 1986) and particularly after fertilization when average leaf tissue NO_3^- concentrations increased to 40–50 μmol g^{-1} FW (Mattsson et al., 2008[2]) 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 the herbage compared to the other grass species (Table 2). On the other hand, *Bromus mollis,* which also showed low leaf tissue NH_4^+ and NO_3^- concentrations, had the highest total N concentration in the leaves and therefore the very lowest C:N ratio.

In the present investigation a clear linear relationship existed between leaf tissue NH_4^+ concentration and Γ across species, but not between substrate N and Γ. Thus, tissue NH_4^+ concentration proved to be a more promising indicator of NH_3 emission potential than substrate N. Similar correlations were also found for apoplastic and leaf tissue NH_4^+ samples over a diurnal course (Herrmann et al., 2008[4]) and over the entire experimental time course with different management events (Mattsson et al., 2008). The ratio between leaf tissue and apoplast NH_4^+ 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 NH_4^+, this ratio was much higher. Prediction of NH_3 exchange potential from leaf tissue NH_4^+ concentrations might therefore be problematic in some cases.

[4]Herrmann, B., Mattsson, M. E., Jones, S. K., Cellier, P., Milford, C., Sutton, M. A., Schjoerring, J. K., and Neftel, A.: Vertical structure and diurnal variability of ammonia emission potential within an intensively managed grass canopy, Biogeosciences Discuss., submitted, 2008.

CC BY

5 Conclusions

We concluded that grass species growing on the same field can differ greatly in the leaf N pools. Apoplastic pH and NH_4^+ concentrations resulted in very different theoretical NH_3 compensation points. Three species (*Lolium perenne, Festuca pratensis* and *Dactylis glomerata*) had NH_3 compensation points and a total abundance high enough to contribute to the NH_3 emission of the whole field. A good correlation between leaf tissue NH_4^+ concentrations and Γ values of most of the different species suggests that the leaf tissue NH_4^+ concentration can be used for predicting the NH_3 exchange potential.

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, 13 740–13 744, 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. Conservation, 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.

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, 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 NH_3 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., 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., Hebbern, 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.
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.
Lohaus, G., Pennewiss, K., Sattelmacher, B., Hussmann, M., and Muehling, K H.: Is the infiltration-centrifugation technique appripriate for isolation of apoplastic fluid? A critical eval-

uation 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 NH_4^+ and pH in an intensively managed grassland, Plant Soil, 238, 97–110, 2002.
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 NH_3 exchange in leaves of *Lolium perenne* and *Bromus erectus* to changes in root N supply, Plant Physiol., 128, 742–750, 2002.
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 medows on a Somerset peat moor, J. Appl. Ecol., 30,321–332, 1993.
Nielsen, K. H. and Schjoerring, J. K.: Regulation of apoplastic NH_4^+ 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. Amer. 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.

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.

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.

Whitehead, D. C.: Grasses: Uptake of nitrogen and effects on morphology and physiology. In: D. C. Whitehead, Ed. Grassland nitrogen. CAB International, UK 16–34, 1995.

Table 1. Life-cycle and Ellenberg indicator values on a scale from 1 to 9 for the 8 most abundant species growing on the experimental site.

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

BGD

5, 2583–2605, 2008

Table 2. Total soluble N concentration, total N concentration and C:N ratio in the plant species occurring at the experimental site before cutting. Means of 4 replicates±SE are shown.

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

BGD

5, 2583–2605, 2008

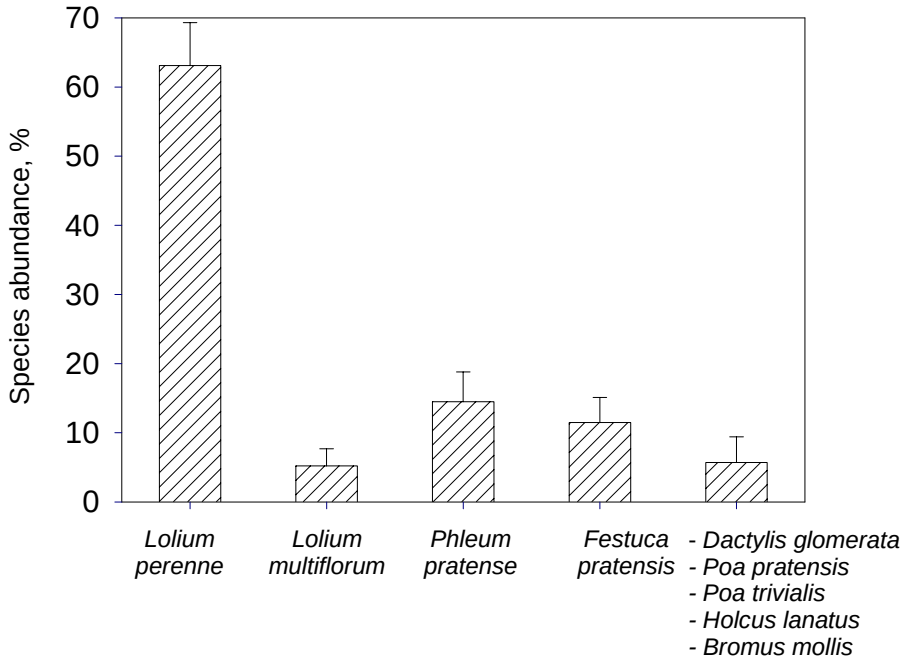

Fig. 1. Plant species abundance at the experimental site assessed by random point contact sampling. Data are means of 20 replicates±SE.

BGD

5, 2583–2605, 2008

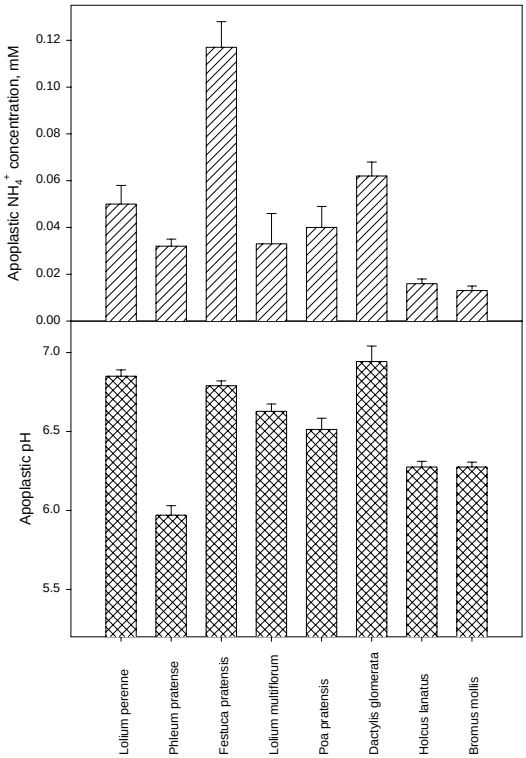

Fig. 2. **(a)** Apoplastic NH_4^+ concentrations and **(b)** apoplastic pH of the different grass species growing on the experimental site before cutting. Values are means±SE for four replicates.

BGD

5, 2583–2605, 2008

Stomatal NH_3 compensation point in different grass species

M. Mattsson et al.

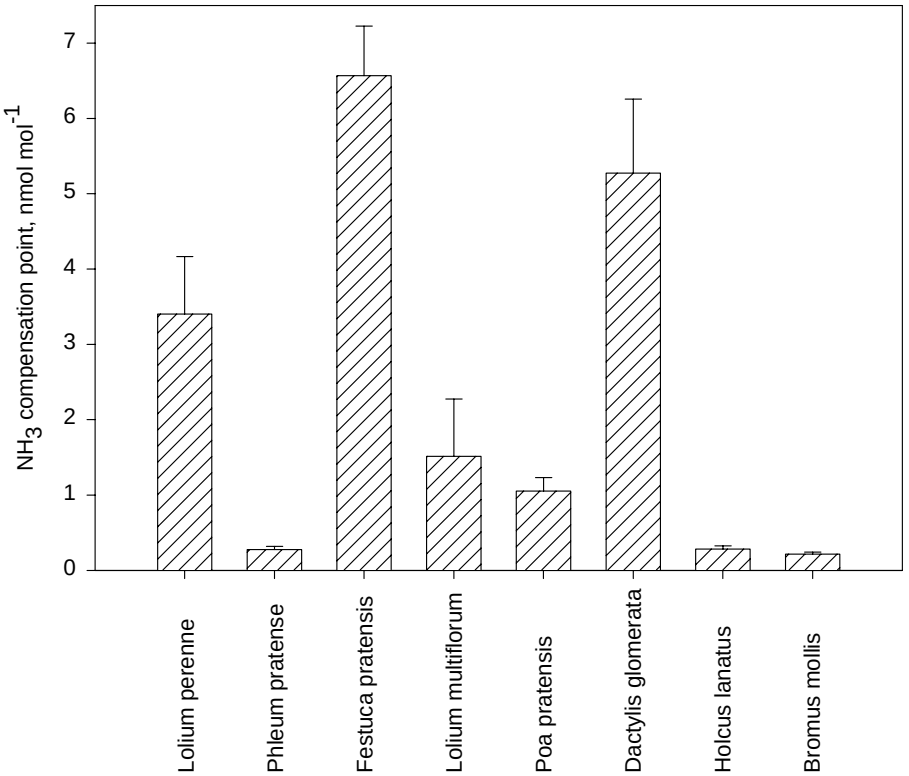

Fig. 3. Compensation points for NH_3 calculated from the apoplastic NH_4^+ and H^+ concentrations of the different species growing on the experimental site before cutting. Values are means±SE for four replicates.

BGD

5, 2583–2605, 2008

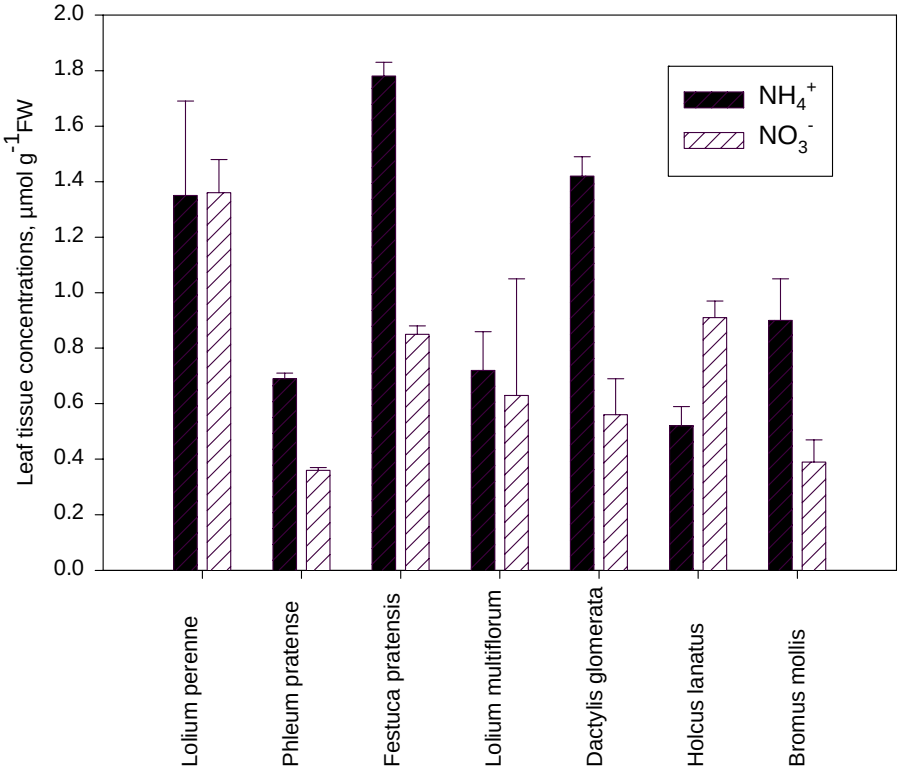

Fig. 4. Bulk leaf tissue NO_3^- and NH_4^+ concentrations of the different species growing on the experimental site before cutting of the grass. Values are means±SE for four replicates.

BGD
5, 2583–2605, 2008

CC BY

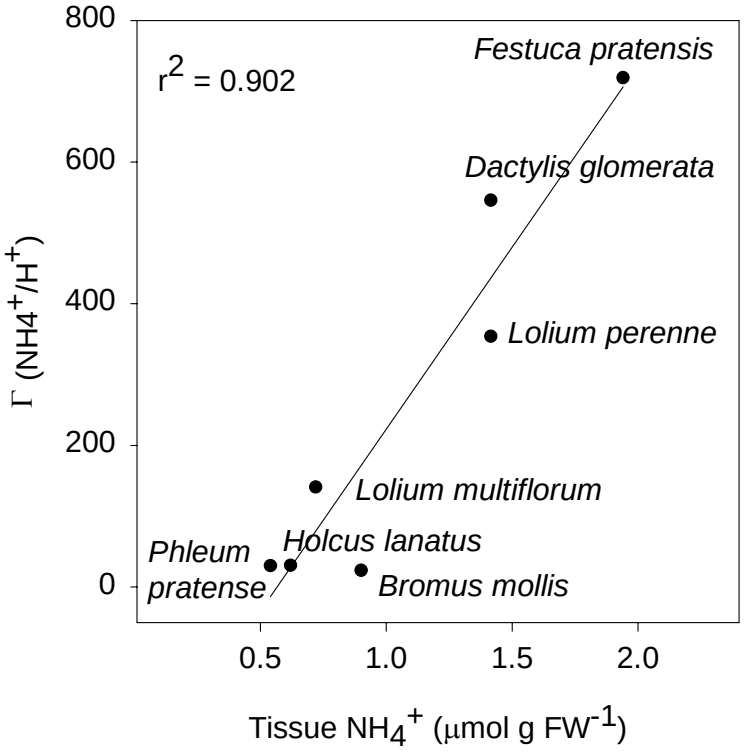

Fig. 5. Correlation between mean bulk leaf NH_4^+ concentration and Γ (apoplastic NH_4^+/H^+) in leaves of the different plant species present on main experimental site.

BGD

5, 2583–2605, 2008