Vertical structure and diurnal variability of ammonia exchange potential within an intensively managed grass canopy

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Abstract

Stomatal ammonia compensation points ($\chi_s$) of grass species on a mixed fertilized grassland were determined by measurements of apoplastic $\text{NH}_4^+$ and $\text{H}^+ \text{H}^+$ in the field. Calculated $\chi_s$-values were compared with in-canopy atmospheric $\text{NH}_3$ concentrations ($\chi_a$) measured by micrometeorological techniques.

Leaf apoplastic $\text{NH}_4^+$ did not significantly differ between intact leaves from different heights above the ground. Bulk leaf $\text{NH}_4^+$ and especially $\text{NO}_3^-$ slightly increased at the bottom of the canopy and these concentrations were very high in senescent plant litter. Calculated $\chi_s$-values were below atmospheric $\chi_a$ at all canopy levels measured, indicating that the grassland was characterized by $\text{NH}_3$ deposition before cutting. This was confirmed by the $\chi_a$ profile, showing the lowest $\chi_a$ close to the ground (15 cm above soil surface) and an increase in $\chi_a$ with canopy height, especially during the night. Neither $\chi_s$ nor $\chi_a$ could be measured close to the soil surface, the litter $\text{NH}_4^+$ material indicated a high potential for $\text{NH}_3$ emission tough.

A diurnal course in apoplastic $\text{NH}_4^+$ was seen in the regrowing grass growing after cutting, with highest concentration around noon. Both apoplastic and tissue $\text{NH}_4^+$ increased in young grass compared to tall grass. Following cutting, in-canopy gradients of atmospheric $\chi_a$ showed $\text{NH}_3$ emission but since calculated $\chi_s$-values of the cut grass were still lower than atmospheric $\text{NH}_3$ concentrations, the emissions could not entirely be explained by stomatal $\text{NH}_3$ loss. High tissue $\text{NH}_4^+$ in the senescent plant material indicated that this fraction constituted an $\text{NH}_3$ source. After fertilization, $\text{NH}_4^+$ increased both in apoplast and leaf tissue with the most pronounced increase in former compared to the latter. The diurnal pattern in apoplastic $\text{NH}_4^+$ was even more pronounced after fertilization and calculated $\chi_s$-values were generally higher, but remained below atmospheric $\text{NH}_3$.  

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1 Introduction

Several investigations have revealed the bidirectional character of NH$_3$ exchange between vegetation and the atmosphere with large fluctuations on annual, seasonal and daily time scales (Sutton et al., 1995; Bussink et al., 1996; Hermann et al., 2001; Horvath et al., 2005; Walker et al., 2006; Sutton et al., 2007). In a non-fertilized managed grassland in The Netherlands, NH$_3$ emission fluxes were frequent (about 50% of the time) during a warm and dry summer period, while in a wet and cool autumn period deposition fluxes dominated (80% of the time; Kruit et al., 2007).

The direction of the NH$_3$ flux between plant leaves and the atmosphere depends on the stomatal NH$_3$ compensation point ($\chi_s$) of leaves, which is the atmospheric NH$_3$ concentration where NH$_3$ emission and deposition are balanced and no net exchange occurs (Farquhar et al., 1980; Husted et al., 1996). In chamber studies $\chi_s$ was shown to be influenced by the N status of the plant (Sharpe and Harper, 1995; Mattsson et al., 1998; Mattsson and Schjoerring, 2002; Sommer et al., 2004) and by environmental factors such as temperature (Mattsson et al., 1997), photosynthetic photon flux density and air humidity (Mattsson and Schjoerring, 1996; Husted and Schjoerring, 1996; Husted et al., 2002).

Measurements of vertical NH$_3$ concentration gradients within a grass/clover canopy (Denmead et al., 1976) and a quackgrass (Agropyron repens L.) canopy (Lemon and van Houtte, 1980) showed a sharp increase of the NH$_3$ concentration towards the soil surface, resulting in a upward NH$_3$ flux from the soil to the base of the grass canopy. Similarly, a more recent study based on the inverse Lagrangian source/sink analysis for an oilseed rape (Brassica napus) canopy also revealed highest NH$_3$ concentrations at the ground level, which was suggested to originate from decomposing litter leaves (Nemitz et al., 2000). This was supported by a very high ammonium NH$_4^+$ concentration measured in senescent plant material from oilseed rape compared to the concentration in intact leaves (Husted et al., 2000). It is not known yet, if corresponding NH$_4^+$ gradients between leaves of different age may occur in perennial grass species.
A diurnal pattern of the NH$_3$ exchange has been observed in *Brassica napus* (Husted et al., 2000), barley (Schjoerring et al., 1993) and grassland (Trebs et al., 2006), with highest NH$_3$ emission rates typically during the daytime and low rates at night. Since diurnal variations in apoplastic NH$_4^+$ and H$^+$ concentrations seem to be small (Husted et al., 2000; van Hove et al., 2002), changes in NH$_3$ emission may be attributed to temperature effects on NH$_3$ solubility and NH$_4^+$ dissociation in the apoplast due to varying canopy temperature during the diurnal course (Husted and Schjoerring, 1996). In addition, fluctuations in leaf surface wetness will affect the NH$_3$ exchange (Walker et al., 2006; Kruit et al., 2007). Diurnal variations of NH$_3$ emission have also been observed over grassland, but correlation between the measured atmospheric $\chi_a$ and $\chi_s$, calculated from flux density measurements, was low (Harper et al., 1996).

The experiment presented here was carried out in May and June 2000 in Braunschweig, Germany and was part of a joint investigation within the EU GRAMINAE project (Sutton et al., 2008$^1$). The aim was to estimate the NH$_3$ exchange potential of the vegetation on a vertical gradient within a fertilized grass canopy and its diurnal fluctuations by means of $\chi_s$. The vacuum infiltration technique for apoplast extraction was directly applied in the field and calculated $\chi_s$ was related to in-canopy NH$_3$ concentrations. It is discussed whether leaf tissue NH$_4^+$ could be a useful indicator of $\chi_s$, since measuring this parameter would be more convenient and less time-consuming than the determination of $\chi_s$.

2 Materials and methods

2.1 Description and management of the measurement site

The measurement site was located near Braunschweig (52°18’N, 10°26’E, 79 m a.s.l.) in Lower Saxony, Germany. The field was 600×300 m in size and consisted of a mixed sward dominated by *Lolium perenne* L., (see footnote 1). It has been a grassland for 4 years, typically receiving 250 kg N ha\(^{-1}\) a\(^{-1}\). Prevailing wind directions were SW to W and E. A farm with 300 cattle and 3000 pigs was located in the W of the field. The field was cut on 29 May and N fertilizer (100 kg N ha\(^{-1}\)) was applied as calcium ammonium nitrate on 5 June. Further details of the experimental set up and site conditions are reported by Sutton et al. (see footnote 1).

2.2 Micrometeorological measurements

Instruments for the measurement of \(\chi_a\) were placed in the centre of the field. \(\chi_a\) was measured continuously online by Mini Wet Effluent Denuders (mini-WEDD), as described by Neftel et al. (1998), connected to a four-channel fluorescent analyzer. Before cutting three of the Mini-WEDDs were placed within the plant canopy and one directly above the canopy. Air flow rates of 200 ml min\(^{-1}\) and 800 ml min\(^{-1}\) were used for the lowest two mini-WEDDs and for the two above, respectively. A liquid flow of 0.12 ml min\(^{-1}\) was used and the detection limit was 0.1 µg NH\(_3\) m\(^{-3}\).

2.3 Sampling of plant material

During the first period of the experiment, a few days before the field was cut, plant material was collected from different layers within the plant canopy and separated into flowers, stems and leaf sheaths and green and brown leaf laminae. The fully developed green leaf laminae were used for apoplast extraction as described below. After the cut it was no longer possible to properly divide plant material into different species. Therefore a mixture of cut leaves from all the species was collected. The plant material was
randomly collected in the field and immediately brought to an adjacent field lab. Some of the leaves were used for extraction directly after sampling and the plant material used for the determination of tissue \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) was immediately frozen in liquid nitrogen and stored at \(-20^\circ\text{C}\).

2.4 Apoplast extraction

Apoplast liquid was extracted by means of vacuum infiltration (Husted and Schjoerring, 1995) modified as follows: Whole leaf laminae or cut leaves were infiltrated with a 280 mM sorbitol solution at a pressure of 16 bar for 5 s followed by vacuum. This procedure was repeated 5 times. After infiltration, leaves were carefully blotted dry, packed into plastic bags and equilibrated for 20 min in the daylight and after that centrifuged for 10 min at \(4^\circ\text{C}\) and 800 g. Concentrations of \( \text{NH}_4^+ \) in the extracted solution were determined by flow injection analysis (FIA) or HPLC analysis (Waters Corp., Milford, USA) using \(\sigma\)-phthalaldehyde (OPA) as reagent as described by Genfa and Dasgupta (1989). Apoplastic pH was measured with a Micro-Combination pH electrode (type 9810, Orion, Beverly, USA). In order to assess cytoplasmic contamination of the apoplast, 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). Cytoplasmic contamination was below 1.5% for all considered plant species.

2.5 Stomatal \( \text{NH}_3 \) compensation points

Estimates of \( \chi_s \) in grass species were obtained from measured apoplastic pH and \( \text{NH}_4^+ \) concentration by the following equation:

\[
\chi_s = K_H \cdot K_d \cdot \left( \frac{\text{NH}_x}{K_d} + [H^+] \right)
\]

where \( \text{NH}_x \) is the \( \text{NH}_4^+ \) and \( \text{NH}_3 \) concentration of the apoplast and \( K_H \) and \( K_d \) the thermodynamic constants of \(10^{-9.25}\) and \(10^{-1.76}\) at \(25^\circ\text{C}\), respectively. \( \chi_s \) was adjusted
to actual canopy temperature by the Clausius-Clapeyron equation:

\[
\ln \left( \frac{\chi_2}{\chi_1} \right) = \frac{\Delta H_{\text{dis}}^0 + \Delta H_{\text{vap}}^0}{R \cdot \left( \frac{1}{T_1} - \frac{1}{T_2} \right)}
\]  

(2)

where \( \chi_1 \) is the \( \text{NH}_3 \) compensation point at the temperature \( T_1 \) and \( \chi_2 \) the \( \text{NH}_3 \) compensation point at the actual canopy temperature \( T_2 \). Canopy \( T \) was measured by an IR Pyranometer. Enthalpies of dissociation of \( \text{NH}_4^+ \Delta H_{\text{dis}}^0 \) and vaporization of \( \text{NH}_3(g) \Delta H_{\text{vap}}^0 \) are 52.21 kJ mol\(^{-1}\) and 34.18 kJ mol\(^{-1}\), respectively, and \( R \) denotes the gas constant \( (8.31 \text{ J K}^{-1} \text{ mol}^{-1}) \).

\( \Gamma \)-values represent a measure of the \( \text{NH}_3 \) exchange potential independent of temperature and are calculated as follows:

\[
\Gamma = \frac{\text{apoplastic } \text{NH}_4^+}{\text{apoplastic } \text{H}^+}
\]  

(3)

2.6 Determination of bulk tissue \( \text{NH}_4^+ \) and \( \text{(NO}_3^-) \)

0.2 g of the frozen plant material was homogenized to powder and was extracted in 2 ml 10 mM formic acid in a cooled mortar containing a little quartz sand. The extract was centrifuged at 25 000 g and 4°C for 10 min. The supernatant was transferred to 500 \( \mu l \) 0.45 \( \mu m \) polysulphone centrifugation filters (Micro VectraSpin, Whatman Ltd., Maidstone, UK) and spun at 5000 g and 4°C for 5 min. \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) of the supernatant was analyzed using a flow injection system (Quik Chem instrument, Lachat Instruments INC, Milwaukee, USA).
3 Results

3.1 Vertical structure of NH₃ exchange potential

Before the field was cut plant material was collected from four different layers, in order to characterise the vertical structure of \([\text{NH}_4^+]\) and \(\text{NO}_3^-\) of the plants. The fully developed canopy was 76 cm high at that stage. Green leaf laminae, which were used for apoplast extraction, were found in all the layers except in the top level (60–70 cm) (Fig. 1). Brown senescent leaves constituted an additional fraction in the lowest canopy layer (0–20 cm), but uncontaminated apoplast liquid could not be obtained from this fraction. Apoplastic \(\text{NH}_4^+\) was highest in young leaves occurring at the upper layer of the plant (Fig. 2A). Yet the difference between the layers was not significant \((p>0.05)\) due to relatively large variability between the replicates, especially in the top canopy layer. Leaf apoplastic pH ranged between 6.3 and 6.6 in all the layers (Fig. 2B). Tissue \(\text{NH}_4^+\) was much higher in brown senescing leaves close to the soil surface compared to green leaves at the same canopy height (Fig. 2C). \(\text{NO}_3^-\) of stems and green leaves decreased with canopy height (Fig. 2D) and was highest in the stems except in the layer closest to the ground where \(\text{NO}_3^-\) was higher in the leaves. Like apoplastic \(\text{NH}_4^+\), \(\chi_s\) did not differ significantly between the different layers and the values were below the measured in-canopy \(\chi_a\) (Fig. 3).

3.2 Diurnal course of NH₃ exchange potential

Before the cut, the most abundant plant species \textit{Lolium perenne} and \textit{Phleum pratense} were selected for determination of the NH₃ exchange potential during a diurnal course. The course of apoplastic \(\text{NH}_4^+\) as well as \(\Gamma (\text{NH}_4^+/\text{H}^+)\) in non senescent green leaves as shown for \textit{Lolium perenne} in Fig. 4A and C did not show any particular pattern whereas apoplastic pH was higher during the night than during the day (Fig. 4B). After the field was cut, apoplastic \(\text{NH}_4^+\) of grass leaves was generally higher and a distinct diurnal course could be seen on the first day, with highest apoplastic \(\text{NH}_4^+\) before
noon and a decrease during the night (Fig. 4A). However, apoplastic NH$_4^+$ remained low on the following day probably because of the lower canopy temperature on the second day compared to the day before. However, the increase in NH$_4^+$ following the cut was more pronounced in the leaf tissue and was also observed on the second day (Fig. 5A). In contrast, NO$_3^-$ seemed to decrease during the day and an increase was observed during the night. (Fig. 5B). Like before the cut, highest apoplastic pH was measured in the night (Fig. 4B). Due to generally lower apoplastic pH of the cut grass mix compared to the grass before cutting, $\Gamma$ was similar before and after the cut (Fig. 4C). After fertilization NH$_4^+$ increased in both the apoplast and the tissue (Fig. 4A and 5A). The diurnal pattern in apoplastic NH$_4^+$ and $\Gamma$ was more pronounced after N application than before. Before fertilization a relatively good correlation was seen between leaf tissue and apoplastic NH$_4^+$, which was significant $p<0.01$ after cutting but not before cutting (Fig. 6). Because apoplastic NH$_4^+$ increased while tissue NH$_4^+$ was rather unaffected after fertilization, the correlation between tissue and apoplastic NH$_4^+$ was very low.

Before the field was cut the vertical profile of $\chi_a$ was predominantly characterised by decreasing $\chi_a$ towards the ground as shown for a diurnal course in Fig. 7. This $\chi_a$ profile would therefore indicate NH$_3$ deposition from the atmosphere to the plant canopy. Calculated $\chi_s$ of both Lolium perenne and Phleum pratense, which corresponded to the upper two $\chi_a$ measuring heights, were below the in-canopy $\chi_a$. The increase in $\chi_a$ during the night was not reflected in $\chi_s$. An inverse $\chi_a$ profile was observed after the canopy had been cut. At the lowest measuring height $\chi_a$ reached 10 µg m$^{-3}$ in the morning and $\chi_a$ decreased with measuring height (Fig. 8). $\chi_a$ was lower during the night than during the day. Accordingly, highest NH$_3$ emission was measured during the day (Milford et al., 2008$^2$). Generally, $\chi_s$ of the cut grass were much lower than

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\( \chi_a \) above the plant canopy. The same direction of the slope of the vertical \( \chi_a \) gradient but higher concentrations during the day were seen after N application (Fig. 9). A typical diurnal pattern with highest concentration around noon was most pronounced after fertilization and was reflected in both calculated \( \chi_s \) and atmospheric \( \chi_a \). Although \( \chi_s \) of the fertilized grass were about five times higher than before fertilization the values were still below atmospheric \( \chi_a \) of the lowest measuring height during the whole diurnal course.

4 Discussion

Application of the vacuum infiltration technique directly in the field enabled an immediate extraction of apoplast liquid and therefore frequent determination of the \( \text{NH}_3 \) exchange potential of the plants during a diurnal course. The measured apoplastic \( \text{NH}_4^+ \) levels before fertilization were about 0.1 mM (Fig. 4A) matching values reported in pastures under similar N conditions by Herrmann et al. (2001) and Loubet et al. (2002). Considerably higher apoplastic \( \text{NH}_4^+ \) concentrations, 0.2 to 0.9 mM, were observed in an intensively managed grassland in The Netherlands throughout the growing season (van Hove et al., 2002). The nitrogen availability in the soil particularly that of ammonium, has a profound influence on apoplastic \( \text{NH}_4^+ \) concentrations as also demonstrated by the increase following fertilisation (Fig. 4A) (Mattsson et al., 2008).

Apoplastic \( \text{NH}_4^+ \) and \( \chi_s \) increased by a factor of two from the bottom to the top of the intact plant canopy (Figs. 2A and 3). Thus, young leaves had a relatively high \( \text{NH}_3 \) emission potential. At all in-canopy levels considered, \( \chi_s \) was below the measured atmospheric \( \chi_a \), indicating that plants acted as \( \text{NH}_3 \) sinks. This was confirmed by the measured \( \text{NH}_3 \) flux which was characterized by \( \text{NH}_3 \) deposition (see footnote Milford et al.) and is in agreement with measurements carried out over a grass/clover canopy (Herrmann et al., 2001).

The \( \text{NH}_3 \) emission measured from the field after the cut could not be totally explained by a raise in \( \chi_s \) of the cut grass. \( \chi_s \) of the senescent plant material either
attached to the stubbles or lying on the ground could, however, not be calculated since apoplastic infiltration of senescent plant material could not be achieved. Yet, very high tissue NH$_4^+$ measured in plant litter, which accounted for about 20% of the total above ground biomass after the cut, indicate that this fraction may represent an important NH$_3$ source. This might explain the NH$_3$ emission measured after cutting, when the litter fraction was not covered by a canopy and no absorption from the intact leaves could occur anymore. Husted et al. (2000) showed that in an oilseed rape field, the plant litter fraction represented an NH$_3$ source, while attached leaves acted as NH$_3$ sinks. Similarly, in a grass/clover crop the highest in-canopy $\chi_a$ was found towards the soil surface (Denmead et al., 1976). In the present investigation atmospheric NH$_3$ could not be measured below 15 cm and therefore NH$_3$ concentration directly above the soil surface is not known. However, using a tissue NH$_4^+$ value for brown leaves as presented in Fig. 2C and a measured pH of 7 (data not shown) would result in $\Gamma$-values for the litter of about 5000. Although this $\Gamma$ value cannot be considered as a direct measure of the effective NH$_3$ emission of plant litter it still indicates a high potential for NH$_3$ emission. Furthermore, NH$_3$ flux measurements carried out in a climate chamber study revealed a NH$_3$ emission of about 10 nmol m$^{-2}$ leaf area s$^{-1}$ from cut senescent leaf material of Lolium perenne (Mattsson and Schjoerring, 2003). This would result in a NH$_3$ emission of about 80 ng m$^{-2}$ s$^{-1}$ using the amount of litter biomass per surface area of 20% of total as measured in the present investigation (David et al., 2008$^3$).

While plant litter emission could explain the measured NH$_3$ emission after the cut it cannot entirely account for the high emission observed after fertilization (see footnote 2). Directly after N application most of the NH$_3$ emission most probably originated from fertilizer particles lying on the ground (Herrmann et al., 2001). Yet, the NH$_3$ emission measured over the following days and its distinct diurnal pattern (see footnote 2) indicate that another NH$_3$ source than fertilizer must be involved. Although $\chi_s$ of the grass

considerably increased after fertilization (Fig. 4C) it still remained below measured atmospheric $\chi_a$ and thus plants should represent an NH$_3$ sink.

A discrepancy between micrometeorological or cuvette studies and the bioassay approach in estimating $\chi_s$ has been observed in several investigations. In most of these studies the bioassay approach yielded smaller estimates of $\chi_s$ compared to the micrometeorological or cuvette measurements (Mattsson et al., 1997; Hill et al., 2001; Mattsson and Schjoerring, 2002). Considering a possible underestimation of $\chi_s$ in the present study, NH$_3$ emission from the plants would become likely, especially after cutting and fertilization around midday, when the ratio between $\chi_a$ and estimated $\chi_s$ was smaller than during the rest of the day. However, the discrepancy between $\chi_a$ and estimated $\chi_s$ was still considerable for most of the collected data, indicating that also after fertilization other NH$_3$ sources might be involved in the NH$_3$ exchange of the canopy.

The diurnal measurements clearly showed that apoplastic NH$_4^+$ may change during the course of the day, with highest values around midday and decreasing concentrations during the night. This pattern was also reflected in $\Gamma$ which is an indicator for the NH$_3$ exchange potential of a plant $g$ but in contrast to $\chi_s$, it is independent of any change in canopy temperature. This is different from observations made in an oilseed rape field, where no diurnal variation in $\Gamma$ existed and where canopy temperature was the only factor influencing $\chi_s$ on a diurnal scale (Husted et al., 2000).

Before fertilization a relatively clear linear relationship existed between leaf tissue NH$_4^+$ and apoplastic NH$_4^+$ (Fig. 6), but this was not the case after fertilization. In addition, the ratio between tissue NH$_4^+$ and apoplastic (NH$_4^+$) was much lower after fertilization compared to before fertilization. These findings differ from studies in a Scottish grassland, where the magnitude of increase in NH$_4^+$ after cutting was similar for the apoplastic and bulk tissue fraction (Loubet et al., 2002). Also in two grass species grown with different N supply the correlation between apoplast and leaf tissue NH$_4^+$ was fairly good (Mattsson and Schjoerring, 2002) while in a wild perennial the same correlation was poor (Hill et al., 2002). The data presented here indicate that
NH$_4^+$ in the tissue and in the apoplast may be regulated independently and thus the tissue NH$_4^+$ can not always be used as an indicator of $\chi_s$.

5 Conclusions

From the present investigation we conclude that the plants of a fully developed grassland acted as NH$_3$ sinks and that NH$_3$ was predominantly deposited to the tall canopy. NH$_3$ emission measured after the cut and after fertilization could not entirely be accounted for by stomatal loss. Yet, elevated tissue NH$_4^+$ and high $\Gamma$-values in especially senescent plant material indicated that NH$_3$ might be emitted from plant litter, which could explain the NH$_3$ emission measured after cutting. Although Mattsson et al. (2008) showed a high inter-species correlation between $\Gamma$ and bulk leaf NH$_4^+$, this comparison shows that there are limitations in this relationship when considering temporal differences for individual species. Specifically, the relationship was shown to change after fertilization, indicating that bulk tissue NH$_4^+$ should only be used as an indicator of $\Gamma$ when calibration specific to current conditions is available.

References


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Fig. 1. Relative contribution of the fresh weight of flowers, stems and leaf sheaths, and green and brown leaf laminae to total plant biomass at different layers within the plant canopy.
Fig. 2. Apoplastic $\text{NH}_4^+$ (A) and pH (B), bulk $\text{NH}_4^+$ (C) and $\text{NO}_3^-$ (D) of grass plants at different heights within the intact canopy on 29 May. For the highest level apoplastic data are means of the dominant species $\text{Lolium perenne}$ and $\text{Phleum pratense}$ weighted for species abundance ($n=8$±$SE$) whereas for the other levels a mixture of all species was considered ($n=4$±$SE$).
Fig. 3. NH₃ profile within the intact grass canopy and calculated mean χ₉₃ of grass leaves on 29 May. For the highest level χ₉₃ data are means of the dominant species Lolium perenne and Phleum pratense weighted for species abundance (n=8±SE) whereas for the other levels a mixture of all species was considered (n=4±SE). NH₃ represent mean concentrations over three days before cutting (10:00 a.m.–16:00 p.m.). The dashed line indicates a potential course of χ₉₃ when calculating χ₉₃ from tissue water pH and NH₄⁺ for the litter fraction.
Fig. 4. Diurnal course of apoplastic $\text{NH}_4^+$ (A), apoplastic pH (B) and $\Gamma$ (apoplastic $\text{NH}_4^+$/H$^+$) (C) in grass leaves before and after cutting and after fertilization. Data are means of 4 replicates±SE and represent a mixture of all species except before fertilization when data represent the most dominant species *Lolium perenne*. 
Fig. 5. Diurnal course of bulk NH$_4^+$ (A) and NO$_3^-$ (B) in grass leaves before and after cutting and after fertilization. Data are means of 4 replicates±SE and represent a mixture of all species.
Fig. 6. Correlation between mean bulk leaf NH$_4^+$ and Γ (apoplastic NH$_4^+$/H$^+$) in leaves of a grass mixture during a diurnal course before and after cutting and after fertilization. **Significance at $p<0.01$. 
Fig. 7. Diurnal course of NH$_3$ flux above the plant canopy (A), in-canopy NH$_3$ gradient and calculated $\chi_{NH_3}$ for the dominant grass species Lolium perenne and Phleum pratense (B) before cutting (26/27 May). The height of the canopy was 70 cm at this stage. $\chi_{NH_3}$ are means of 4 replicates±SE. The dark period is indicated by the shaded area.
Fig. 8. Diurnal NH₃ gradient above the canopy and calculated χ_NH₃ for grass stubbles after cutting and prior to fertilization (4/5 June). χ_NH₃ are means of 4 replicates ± SE. The dark period is indicated by the shaded area.
Fig. 9. Diurnal NH$_3$ gradient above the canopy and calculated $\chi_{\text{NH}_3}$ for grass stubbles 7 days after fertilization (12/13 June). $\chi_{\text{NH}_3}$ are means of 4 replicates±SE. The dark period is indicated by the shaded area.