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N budget and NH_3 exchange of a grass/clover crop at two levels of N application

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Abstract

Atmospheric ammonia (NH_3) exchange during a single growing season was measured over two grass/clover fields managed by cutting and treated with different rates of mineral nitrogen (N) fertilizer. The aim was to quantify the total NH_3 exchange of the two systems in relation to their N budget, the latter was split into N derived from symbiotic fixation, from fertilization, and from the soil. The experimental site was located in an intensively managed agricultural area on the Swiss plateau. Two adjacent fields with mixtures of perennial ryegrass (*Lolium perenne* L.), cocks foot (*Dactylis glomerata* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) were used. These were treated with either 80 or 160 kg N ha^{-1} applied as NH_4NO_3 fertilizer in equal portions after each of four cuts. Continuous NH_3 flux measurements were carried out by micrometeorological techniques. To determine the contribution of each species to the overall NH_3 canopy compensation point, stomatal NH_3 compensation points of the individual plant species were determined on the basis of NH_4^+ + NH_3 (NH_x) concentrations and pH in the apoplast. Symbiotic N_2 fixation was measured by the ^{15}N dilution method.

In the field with the lower rate of mineral N application, the clover fraction was higher, and a higher symbiotic N_2 fixation rate completely compensated for the lower mineral N input. During the measurement period, except after N fertilization, NH_3 concentration ([NH_3]) above the canopy was between 3 and 4 μg m^{-3}, which was generally higher than the measured canopy NH_3 compensation point. Thus, deposition from the atmosphere to the grass/clover canopy was predominant, and the system acted as net sink for NH_3. The total amount of N emitted as NH_3 was slightly higher for the high N treatment compared to the low N treatment but accounted for less than 1% of the N removed by cutting in both treatments. The results show that net NH_3 emission from the frequently cut grass/clover field was restricted to short periods after ammonium nitrate application, and that on a seasonal basis fertilizer N and N derived from N_2 fixation had equal effects on the exchange of NH_3 between the canopy and the atmosphere.

Abbreviations: χ_{NH3} – stomatal NH_3 compensation point

Introduction

Agricultural production systems are considered to be a major source of atmospheric ammonia (NH_3). NH_3 has important effects on atmospheric chemistry (Dentener and Crutzen, 1994) and potentially severe impacts on the stability of sensitive ecosystems resulting from soil acidification (Gundersen and Rasmussen, 1990) and eutrophication (Bobbink et al., 1992; Sutton et al., 1992). A major part of the global NH_3 emissions originates from animal husbandry and from the application of mineral fertilizers (Buijsman et al., 1987). Global estimates of NH_3 emissions from vegetation range between 5.2 Tg N a^{-1} (Dentener

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and Crutzen, 1994) and 10 Tg N a^{-1}(Schlesinger and Hartley, 1992), which accounts for about 16% of the total anthropogenic NH_3 volatilization (Dentener and Crutzen, 1994).

Atmospheric NH_3 exchange may represent a significant component of the N-balance of agricultural ecosystems. Cumulative annual NH_3 emission by crops of up to 15 kg N ha^{-1} have been reported, for instance, for wheat (Harper et al., 1987). In more recent studies, lower cumulative NH_3 emissions in the range 1 – 5 kg N ha^{-1} a^{-1} have been found for different crops (e.g. Schjoerring and Mattsson, 2001; Yamulki et al., 1996). However, the net NH_3 exchange of crops is influenced by the amount and timing of mineral N application (Mattsson et al., 1998; Schjoerring, 1991; Sharpe and Harper, 1995). During the year, NH_3 exchange of crops is characterized by alternating periods of emission and deposition of NH_3; thus, crops can act both as a sink or a source of NH_3 (Langford and Fehsenfeld, 1992; Morgan and Parton, 1989; Schjoerring et al., 1993; Sutton et al., 1993). NH_3 emission takes place when the atmospheric NH_3 concentration is below the canopy NH_3 compensation point, while NH_3 uptake occurs in the opposite case. The canopy NH_3 compensation point mainly reflects χ_{NH3} of individual leaves, which is the NH_3 concentration at which NH_3 absorption and -emission are balanced, resulting in a zero net flux of NH_3 (Farquhar et al., 1980). χ_{NH3} is determined by the NH_x concentration and pH in the apoplast; hence, χ_{NH3} mainly depends on the physiological and N status of the plant (Farquhar et al., 1980; Mattsson et al., 1998). It varies during ontogeny and among plant species or cultivars (Farquhar et al., 1980; Husted et al., 1996a).

Less is known about the NH_3 exchange of fertilized grasslands; net emission rates below 1 kg N ha^{-1} have been found for grasslands in Scotland treated with NH_4NO_3 (Sutton et al., 1993), and Ryden et al. (1987) reported up to 1.6 kg N ha^{-1} with NH_4NO_3 fertilizer applied at a rate of 100 kg ha^{-1}. Net NH_3 emissions may vary strongly with soil pH, soil type, and climatic conditions (van der Weerden and Jarvis, 1997). In grasslands a considerable amount of N may be added by symbiotic N_2 fixation of legumes. For instance, Boller and Nösberger (1987) reported annual N inputs via symbiosis of over 300 kg N ha^{-1} a^{-1} in a grass/clover mixture. This additional N input is important with respect to the system N balance and could thus affect the NH_3 exchange of grasslands. Since N fixation is inhibited by soil mineral N (Streeter, 1988), a higher N input from fixation is expected at low compared to high N application levels, and a significant amount of N may be transfered from legumes to grass species mainly by decomposition of legume tissue (Broadbent et al., 1982; Boller and Nösberger, 1987). However, it is not clear whether N from symbiotic fixation influences χ_{NH3} to the same extent as N derived from mineral fertilizers.

The objective of the present study was to quantify the NH_3 exchange of a grass/clover canopy at two levels of mineral N fertilization in relationship to the N budget for harvested plant biomass, and to quantify the contribution of symbiotic N_2 fixation to both the N budget and the seasonal net NH_3 exchange.

Materials and methods

Experimental site

The experimental site Kerzersmoos (436 m a.s.l.) was located in an intensive agricultural area on the Swiss plateau, 25 km to the west of Bern (46° 59′ 42″N, 7° 11′ 02″E). A field of 200 m × 40 m with a 1-year-old mixed sward with perennial ryegrass (*Lolium perenne* L. var. Bastion), cocksfoot (*Dactylis glomerata* L. var. Pizza), white clover (*Trifolium repens* L. var. Seminole) and red clover (*Trifolium pratense* L. var. Marino) was used. The soil type was a Mollic Gleysol with a pH between 7.5 and 8.0. During the growing season of 1999 the sward was mown four times to produce either silage or hay. Dates of cutting were 23 May, 1 July, 12 August and 7 October, 1999. Fertilizer N was applied as NH_4NO_3 (27.5%) after each cut. The field was split into two parts with one half receiving 20 kg N ha^{-1} (low N) and the other half receiving 40 kg N ha^{-1} (high N). This resulted in a total N application of 80 kg N ha^{-1} a^{-1} and 160 kg N ha^{-1} a^{-1} for low N and high N, respectively.

Dry matter yield, N content and species composition

At each cutting date the plant biomass in 3 subplots (2 × 10 m) of each N treatment was clipped 5 cm above the soil surface and weighed. Representative samples of each subplot were dried at 65 °C for 48 h before dry weight determination. The dry weight was used to calculate total forage yield (kg dry matter ha^{-1}).

To determine the species composition of the sward, plant material was harvested in 8 randomly selected subplots (0.5 × 0.5 m) of each N treatment. The material was separated by species, dried at 65 °C for 48 h, and the dry weight was determined for each fraction.

Total N content was determined as described below for ^{15}N analysis.

Measurement of χ_{NH3}

In order to determine χ_{NH3} in the field, leaves of the dominant species were randomly collected in both N treatments. Sampling was performed on selected days during the whole observation period between the end of April and the beginning of September. Only a small fraction of leaves reached the stage of senescence, and these were excluded from the measurements. Leaves were packed in plastic bags and immediately brought to an adjacent field laboratory for analysis. Apoplast liquid was extracted by means of vacuum infiltration according to Husted and Schjoerring (1995). Clover leaves were separated into leaflets, and leaf lamina of grasses were cut into segments of about 5 cm length. Leaves were infiltrated with a 280 m*M* 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 and centrifuged for 10 min at 4 °C at 1800 *g* and 800 *g* for clover and grass species, respectively. Different centrifugation velocities for clover and grass species were necessary to obtain a sufficient amount of apoplast liquid for the analysis with minimal cytoplasmic contamination. Concentrations of NH_x in the extracted solution were determined by flow injection analysis (FIA) using *o*-phthalaldehyde (OPA) as reagent (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 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).

Stomatal NH_3 compensation points were calculated on the basis of the equilibrium between aqueous NH_3 and NH_4^+ concentrations and the NH_3 concentration in the gaseous phase of the apoplast and were adjusted to canopy temperature by the Clausius–Clapeyron equation (Husted and Schjoerring, 1996b). Apoplastic NH_x concentrations were not corrected for dilution because the rapid re-circulation of NH_x via the plasmalemma into the apoplast was assumed to maintain apoplast NH_x homeostasis after infiltration (Nielsen and Schjoerring, 1998). Ionic strength of the apoplast was measured with a portable conductivity meter (CDH-42, Omega Engineering, Inc., Stamford, USA). To determine the seasonal course, χ_{NH3} in each dominant plant species was determined 4 or 5 times between 10:00 h and 15:30 h on selected days. Canopy NH_3 compensation points were calculated from χ_{NH3} of the individual plant species and the relative contribution of each species to the composition of the mixture.

Micrometeorological measurements

Instruments to measure atmospheric [NH_3] and NH_3 fluxes were placed in the centre of the field, the high N treatment lying to the east and the low N treatment to the west of the installations. Main wind directions were either from west or from east. NH_3 concentrations were measured continuously on-line by Mini Wet Effluent Denuders (mini-WEDD), as described by Neftel et al. (1998), connected to a four-channel fluorescent analyzer. Mini-WEDDs were placed 0.1 m above the plant canopy in both N treatments and at a height of 2 m in the centre between the two N treatments. An air flow rate of 600 ml min^{-1} and a liquid flow rate of 0.12 ml min^{-1} were used. The detection limit was 0.1 μg NH_3 m^{-3}.

For the determination of NH_3 fluxes the gradient-diffusion technique was used (Monteith and Unsworth, 1990). Only periods with prevailing winds from east or from west were considered, corresponding to the field section with the high N or the low N treatment, respectively. NH_3 concentrations and NH_3 fluxes were averaged over 30 min. To determine the net NH_3 exchange, NH_3 fluxes were integrated over the whole measuring period, including both daytime and nighttime hours. Canopy temperature was continuously measured with a IR-thermometer (KT 15, lens K6, Heimann, Wiesbaden, Germany).

Determination of symbiotic N_2 fixation

N_2 fixation during each of the four regrowth periods was determined with the enriched ^{15}N isotope dilution method (McAuliffe et al., 1958). In each N treatment, 6 plots of 2.25 m^2 with the same clover/grass mixture as the rest of the field were used. N was applied as a $^{15}NH_4{}^{15}NO_3$ solution followed by sufficient watering. The ^{15}N atom%excess was 0.5% for low N and 0.25% for high N. The plots were first labelled in September 1998 and after each of the four cuts during 1999. Above-ground plant material was harvested from the central part (0.5 × 0.5 m) of each plot, separated by species, dried for 48 h at 65 °C, and ground to a fine powder by an ultra-centrifugal mill (Retsch, Arlesheim, Switzerland). The samples

were re-dried for 12 h at 35 °C and prepared for analysis according to the procedure described by Zanetti et al. (1996). Analysis of the samples for ^{15}N and total N concentration was carried out by a continuous-flow mass spectrometer at the Stable Isotope Facility, University of Davis, CA, USA.

N derived from N_2 fixation (N_{fix}) for the two clover species was calculated using the following equation (McAuliffe et al., 1958):

$$N_{fix} = \left(1 - \frac{^{15}Natom\%excess_{clover}}{^{15}Natom\%excess_{reference\ plant}}\right) \times N_{tot}$$

^{15}N atom%excess in the reference plant was calculated as the average of the two dominant grass species *Dactylis glomerata* and *Lolium perenne*. N_{tot} represents the total N content ha^{-1} of the clover species and was derived from the total dry matter yield ha^{-1}, plant species composition and N content measured by continuous-flow mass spectrometry, as described above.

Calculation of N budgets

The difference between the ^{15}Natom%excess of the fertilizer and the reference plants was used to calculate N derived from fertilizer for the individual grass species (N_{fert}), according to Vose and Victoria (1986):

$$N_{fert} = \left(\frac{^{15}Natom\%excess_{plant}}{^{15}Natom\%excess_{Fertilizer}}\right) \times N_{tot}$$

For clover species the following equation was used:

$$N_{fert} = \left(\frac{^{15}Natom\%excess_{(DG)} + ^{15}Natom\%excess_{(LP)}}{^{15}Natom\%excess_{fertilizer} \times 2}\right) \times (N_{tot} - N_{fix})$$

where ^{15}Natom%excess$_{(DG)}$ and ^{15}Natom%excess$_{(LP)}$ represent the ^{15}Natom%excess of the two reference plants *Dactylis glomerata* and *Lolium perenne*, respectively.

N derived from the soil for grasses was calculated as:

$$N_{soil} = \left(1 - \frac{^{15}Natom\%excess_{plant}}{^{15}Natom\%excess_{fertilizer}}\right) \times N_{tot}$$

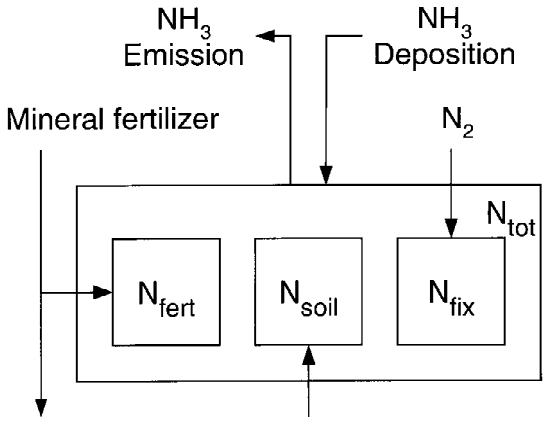

Figure 1. Components considered in the calculated N budget of the total above ground plant biomass of the grass/clover sward (N_{fert}, N derived from mineral fertilizer; N_{fix}, N derived from N_2 fixation; N_{soil}, N derived from soil; N_{tot}, total N content of plant material).

and for clover species:

$$N_{soil} = \left(1 - \frac{^{15}Natom\%excess_{(DG)} + ^{15}Natom\%excess_{(LP)}}{^{15}Natom\%excess_{fertilizer} \times 2}\right) \times (N_{tot} - N_{fix})$$

The individual components of the N budget for harvested biomass are depicted in Figure 1. Adding up N_{fix}, N_{fert} and N_{soil} of the individual plant species yielded the total N budget.

Results

Dry matter yield, N concentration, and species composition

Total annual forage yield in both N treatments was about 14 t dry matter ha^{-1} (Table 1). Total N concentration (in% dry weight) of both clover and grass species did not differ between the N treatments. N concentration was generally higher in clover (3.2–4.1%) than in grass species (2.0–3.4%). As compared to the first and second cut, higher N concentrations for the last two cuts were measured in the grass species, but not in the clover species (Table 2).

The legume fraction of the grass/clover mixture increased about two-fold after the first cut, and was higher at low N compared to high N during the whole measuring period. In the low N treatment, this fraction accounted for almost 70% of the species composition,

Table 1. Dry matter yield (t ha^{-1}) of a mixed clover/grass sward with N application levels of 20 kg N ha^{-1} (low N) or 40 kg N ha^{-1} (high N) after each cut. Means of 3 replicates ± SE are shown

Cut 1		Cut 2		Cut 3		Cut 4		Total	
low N	high N	low N	high N	low N	high N	low N	high N	low N	high N
6.0 ± 0.5	6.6 ± 0.3	1.7 ± 0.3	1.6 ± 0.2	3.7 ± 0.1	3.4 ± 0.3	2.7 ± 0.2	2.8 ± 0.1	14.1 ± 0.6	14.4 ± 0.5

Table 2. Total N concentration (% dry weight) in above-ground plant biomass of the dominant plant species of a mixed grass/clover sward. N application levels were 20 kg N ha^{-1} (low N) and 40 kg N ha^{-1} (high N) after each cut. Means of 8 replicates ± SE are shown

Species	N_{tot} (%)							
	Cut 1		Cut 2		Cut 3		Cut 4	
	low N	high N	low N	high N	low N	high N	low N	high N
Trifolium pratense	3.33 ± 0.15	3.56 ± 0.24	3.58 ± 0.04	3.42 ± 0.08	3.37 ± 0.05	3.24 ± 0.12	3.64 ± 0.12	3.62 ± 0.07
Trifolium repens	4.01 ± 0.10	3.67 ± 0.12	3.70 ± 0.09	4.09 ± 0.16	3.66 ± 0.06	3.39 ± 0.15	3.80 ± 0.09	3.57 ± 0.09
Dactylis glomerata	1.98 ± 0.11	2.12 ± 0.10	2.10 ± 0.05	2.08 ± 0.10	2.85 ± 0.10	2.61 ± 0.03	2.95 ± 0.10	2.84 ± 0.10
Lolium perenne	2.51 ± 0.22	2.60 ± 0.14	2.38 ± 0.13	2.92 ± 0.15	3.31 ± 0.14	3.14 ± 0.06	3.26 ± 0.12	3.40 ± 0.09

whereas at high N the average was less than 50% (Table 3). Red clover (*T. pratense* L.) was much more abundant compared with white clover (*T. repens* L.), and among the grass species *D. glomerata* was more abundant than *L. perenne*.

Symbiotic N_2 fixation

During all regrowth periods, except the last one, and for both clover species, symbiotic N_2 fixation was significantly higher at low N compared to high N (Table 4). The fraction of N derived from symbiotic N_2 fixation in both clover species ranged between 75 and 92% of the total N content for low N, and between 55 and 83% for high N.

NH_3 compensation point (χ_{NH3})

For the determination of stomatal NH_3 compensation points in the field, the vacuum infiltration technique for the extraction of apoplastic fluid was successfully adapted to the dominant plant species. Cytoplasmic contamination was below 1% in all species (Table 5).

Daily mean apoplastic NH_x concentration was generally higher in clover than in grasses, and except for *T. repens*, it was always below 0.1 m*M* (Figure 2A). However, χ_{NH3} of clover species and χ_{NH3} of *D. glomerata* were similar (Figure 2C). The difference in

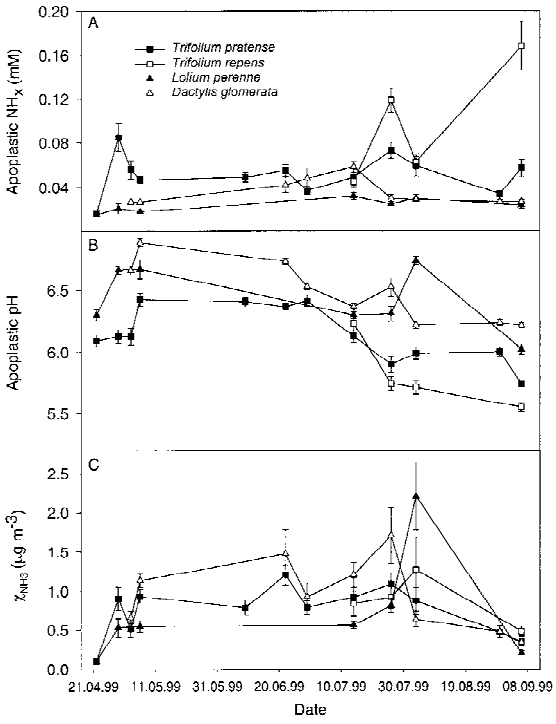

Figure 2. Seasonal course of apoplastic NH_x concentration (A), apoplastic pH (B), and χ_{NH3} (C) of the dominant plant species in a mixed grass/clover sward at the high N application level (160 kg N ha^{-1} a^{-1}). Means of 4 replicates ± SE are shown.

Table 3. Plant species composition expressed as dry weight fraction (%) of a mixed grass/clover sward at N application levels of 20 kg N ha^{-1} (low N) and 40 kg N ha^{-1} (high N) after each cut. Means of 8 replicates ± SE are shown

Species	Species abundance (%)							
	Cut 1		Cut 2		Cut 3		Cut 4	
	low N	high N	low N	high N	low N	high N	low N	high N
Trifolium pratense	26.8 ± 7.1	19.1 ± 5.0	53.8 ± 5.5	39.1 ± 7.2	56.7 ± 5.3	44.0 ± 7.7	41.2 ± 6.0	24.5 ± 5.5
Trifolium repens	2.3 ± 0.8	0.9 ± 0.5	7.1 ± 1.7	6.4 ± 3.8	9.5 ± 3.3	8.4 ± 4.8	19.5 ± 5.0	11.4 ± 3.9
Dactylis glomerata	41.0 ± 6.7	49.8 ± 6.4	24.6 ± 4.4	32.7 ± 8.8	26.9 ± 3.0	35.4 ± 8.2	23.9 ± 2.8	43.8 ± 8.2
Lolium perenne	24.8 ± 4.9	29.5 ± 3.3	13.0 ± 2.1	19.1 ± 5.5	4.9 ± 0.7	9.8 ± 2.1	9.5 ± 2.1	16.5 ± 4.6
Others	5.0 ± 1.5	0.8 ± 0.3	1.4 ± 0.4	2.7 ± 0.8	1.9 ± 0.3	2.5 ± 0.5	5.9 ± 1.6	3.8 ± 0.4

Table 4. Fraction of N derived from N_2 fixation (% of total N content) for *T. pratense* and *T. repens* in a mixed clover/grass sward. N application levels were 20 kg N ha^{-1} (low N) and 40 kg N ha^{-1} (high N) after each cut. Means of 6 and 5 replicates ± SE are shown for *T. pratense* and *T. repens*, respectively

Species	N from N_2 fixation (%)							
	Cut 1		Cut 2		Cut 3		Cut 4	
	low N	high N	low N	high N	low N	high N	low N	high N
Trifolium pratense	92 ± 1	83 ± 1	77 ± 2	72 ± 1	86 ± 1	75 ± 2	80 ± 2	73 ± 6
significance	***		(*)		**			
Trifolium repens	92 ± 1	81 ± 3	84 ± 1	55 ± 4	86 ± 3	74[a]	75 ± 6	78 ± 1
significance	*		*					

(*), **, *** indicate significant difference between low N and high N at $p<0.1$, $p<0.05$, $p<0.01$ and $p<0.001$.
[a] n=1.

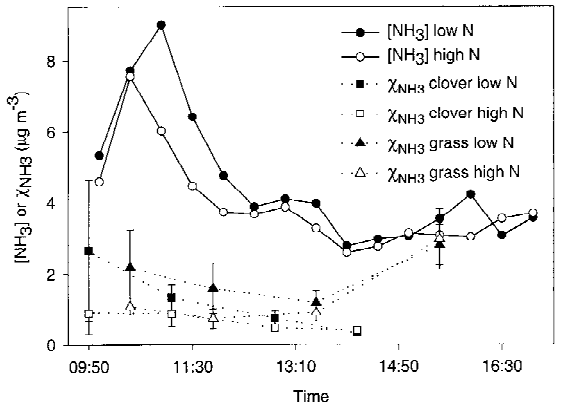

Figure 3. Diurnal course of χ_{NH3} in clover (*T. pratense, T. repens*) and grass species (*D. glomerata*,*L. perenne*), and NH_3 concentration above the grass/clover canopy for 4 August 1999. N application levels were 20 kg N ha^{-1} (low N) or 40 kg N ha^{-1} (high N) after each cut . χ_{NH3} are means ± SE for grass or clover species (n=4).

χ_{NH3} between the species remained relatively stable in the course of the first three months, with highest χ_{NH3}during summer. In early spring and in autumn χ_{NH3} was lower and the difference among the species was smaller. During the measurement period, χ_{NH3} ranged between 0.5 and 2.5 μg m^{-3}. Diurnal variations showed no consistent pattern of χ_{NH3} across all selected days (Figure 3). For all plant species, high N did not result in systematically higher values of χ_{NH3} compared with low N. Surprisingly, even higher χ_{NH3} for low N have been observed on some days, as illustrated by the data collected on 4 August. Over the whole measuring period, differences in χ_{NH3} between the two N treatments fluctuated around zero.

NH_3 fluxes

[NH_3] above the canopy was consistently higher than χ_{NH3}, and higher than the canopy NH_3 compensation points. This is shown for one selected day in

Table 5. Ionic strength and cytoplasmic contamination of the apoplast in the dominant plant species. Data are means of 4 replicates ± SE, each consisting of 8 leaves (clover species) or 10 leaf lamina (grass species)

Measurement	Plant species			
	Trifolium repens	*Trifolium pratense*	*Dactylis glomerata*	*Lolium perenne*
Ionic strength of apoplast (m*M*)	3.8 ± 0.2	3.9 ± 0.1	9.8 ± 1.0	12.6 ± 0.6
Cytoplasmic contamination (%)[a]	0.68 ± 0.19	0.41 ± 0.15	0.29 ± 0.20	0.23 ± 0.17

[a] MDH activity in apoplastic fluid relative to activity in bulk leaf extract.

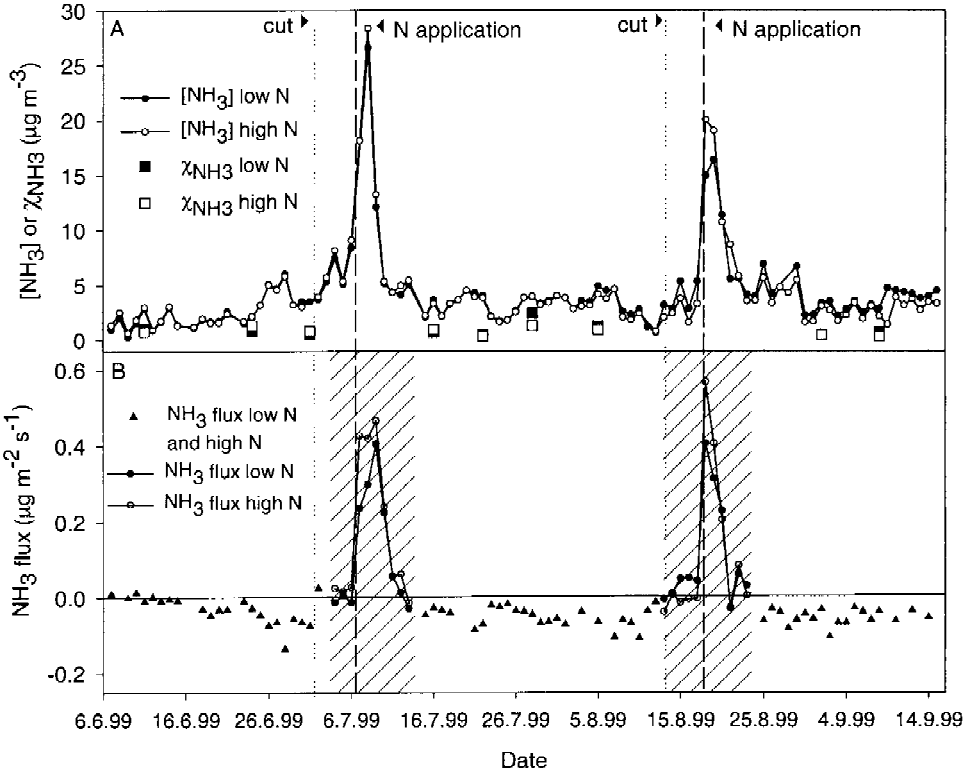

Figure 4. Seasonal course of canopy NH_3 compensation points and NH_3 concentrations above the grass/clover canopy (A), and NH_3 fluxes (B). N application levels were 20 kg N ha^{-1} (low N) or 40 kg N ha^{-1} (high N) after each cut. Data represent the average concentrations between 10.00 h and 17.00 h. NH_3 fluxes are representative for both low N and high N, except after the cut and a few days after fertilization, when NH_3 fluxes are calculated separately for low N and high N (shaded area).

Figure 3, and during three regrowth periods in Figure 4A. On the average, $[NH_3]$ was between 2 and 4 μg m^{-3}, except after fertilization when $[NH_3]$ was as high as 30 μg m^{-3}. Since χ_{NH3} and $[NH_3]$ did not differ significantly between low N and high N over the whole measuring period, except after cutting and fertilization (shaded area in Figure 4B), NH_3 fluxes were assumed to be the same above the western and the eastern field section, corresponding to low N and high N, respectively. Thus, starting a few days after fertilization NH_3 fluxes during the regrowth period were calculated based on $[NH_3]$ measured at 2 m height in the centre of the field and $[NH_3]$ measured above the canopy in either the western or the eastern field section, depending on the wind direction. This flux was regarded as representative for both field sites. Before, i.e. after cutting and until a few days after fertilization (Figure 4B, shaded area), NH_3 fluxes were determined separately for both field sections using $[NH_3]$ measured at 2 m and $[NH_3]$ measured in parallel at the level of the canopy in both sections.

For all regrowth periods, the NH_3 exchange was dominated by deposition of NH_3 from the atmosphere to the plant canopy. Net NH_3 emissions could be

measured following the cuts, and emission rates were higher after the first cut (data not shown) when the plant material was left to dry on the ground, compared with the other cuts when it was removed to produce silage. Highest NH_3 emission rates were detected following the application of N fertilizer (Figure 4B).

N budget

A higher symbiotic N_2 fixation in the low N treatment resulted in an equal N budget for both N treatments, with a total N yield of about 400 kg N ha^{-1}. Total N emission, mainly attributed to periods after fertilization, was higher at high N compared to low N. Nevertheless, for both N treatments total loss of NH_3–N accounted for less than 1% of the N removed by cutting. Over the measuring period, total NH_3 emissions in both treatments were exceeded by NH_3 deposition. The resulting net NH_3 deposition was about 1 kg N ha^{-1} in both N treatments (Table 6).

Discussion

In the present study, the N budget for the harvested plant biomass did not differ between the low N and the high N treatment because the higher symbiotic N_2 fixation rate completely compensated for the lower mineral N input at low N. Increased inputs of N *via* symbiotic N_2 fixation at lower N application is well known (Boller and Nösberger, 1987; Minchin et al., 1986; Silsbury et al., 1986). Along with the balanced N budgets, canopy NH_3 compensation points and NH_3 exchange did not differ between the two N treatments, thus indicating that on a seasonal scale N_2 fixation contributed to the canopy NH_3 compensation point to the same extent as N derived from mineral N fertilization.

In both N treatments, differences in the N status among the plant species were measured. As shown for high N, total N concentrations (Table 2) and apoplastic NH_x concentrations (Figure 2A) were higher in clover than in grass species, but apoplastic pH was lower in clover (Figure 2B). A small difference in apoplastic pH effectively influences the ratio between NH_3 and NH_4^+ in the apoplast and, consequently, χ_{NH3}. As a result, values for χ_{NH3} in the two most dominant species *D. glomerata* and red clover were similar in both N fertilizer treatments. Since the canopy was dominated by these two species, the resulting canopy NH_3 compensation points did not differ between the two N treatments, despite a larger fraction of red clover in the low N treatment. In white clover, the low apoplastic pH resulted in low χ_{NH3}, but the biomass fraction of white clover was small (Table 3) and the individual plants were mostly present in the lower portion of the canopy. Thus, χ_{NH3} in white clover contributed little to the canopy NH_3 compensation point. χ_{NH3} was also lower in ryegrass than in the other species at both low and high N. In all species χ_{NH3} was not influenced by whether N was derived from N_2 fixation or mineral N, since no difference in the apoplastic NH_x concentration nor in χ_{NH3} between low N and high N could be measured.

χ_{NH3} of all species remained low throughout the measuring period, ranging between 0.5 and 2.5 μg m^{-3}which corresponds to about 0.7 and 3.5 nmol mol^{-1}, respectively. In comparison, field measurements revealed maximum χ_{NH3} exceeding 20 nmol mol^{-1} in *Triticum aestivum* (Harper et al., 1987), 1 – 9 nmol mol^{-1} in *Hordeum vulgare* (Schjoerring et al., 1993), or between 5 and 8 nmol mol^{-1} in *Glycine max* (Lemon and van Houtte, 1980). The wide range of χ_{NH3} reflects the variable influence of the plant N status or of plant age across these studies. It was shown for several species that χ_{NH3} typically increases with leaf age, and that χ_{NH3} is often highest during the development of fruits or during senescence (Farquhar et al., 1980; Harper et al., 1983; Husted et al., 1996a). Most arable crops are harvested after maturation and when seed production is complete. Thus, the present situation with a frequently cut grass/clover sward clearly differed from the situation in arable crops; only a small fraction of leaves reached the senescent stage during the short regrowth periods, and the plants were harvested before the development of seeds. Hence, N taken up was efficiently used for the production of new plant biomass. This may explain why χ_{NH3} was generally lower than observed in arable crops.

During most of the measuring period, except after N fertilization events, [NH_3] above the canopy was between 2 and 4 μg m^{-3}, and it was generally higher than the measured canopy NH_3 compensation points (Figure 4A). Therefore, when excluding short periods after cutting and fertilization, the system was dominated by NH_3 deposition (Figure 4B). The small NH_3 emission measured after cutting could be due to either NH_3 liberated from plant material lying on the ground, or from stubble with a reduced N demand due to low photosynthetic activity. High NH_3 emission measured after the application of mineral N fertilizer is

Table 6. N budget for harvested above-ground plant biomass, separated into N from N_2 fixation (N_{fix}), N from soil (N_{soil}), N from fertilizer (N_{fert}), and N yield (N_{tot}), in relation to NH_3 deposition (NH_3–N dep), NH_3 emission (NH_3-N em) and net NH_3 exchange (net NH_3-N ex) for a mixed grass/clover sward. N application levels were 20 kg N ha^{-1} (low N) and 40 kg N ha^{-1} (high N) after each cut. Data represent the sum of the N fractions (N_{fix}, N_{soil}, N_{fert} and N_{tot}) of the individual plant species

	N yield (kg N ha^{-1})									
	Cut 1		Cut 2		Cut 3		Cut 4		Cut 1–4	
	low N	high N	low N	high N	low N	high N	low N	high N	low N	high N
N_{fix}	54 ± 14	39 ± 10	29 ± 6	18 ± 4	73 ± 7	43 ± 8	47 ± 7	27 ± 5	203 ± 18	127 ± 15
N_{soil}	66 ± 9	77 ± 8	16 ± 2	17 ± 3	34 ± 3	33 ± 5	31 ± 3	36 ± 5	148 ±11	164 ± 11
N_{fert}	25 ± 4	50 ± 5	6 ± 1	11 ± 2	13 ± 1	23 ± 4	9 ± 1	23 ± 3	53 ± 4	107 ± 8
N_{tot}	145 ± 20	166 ± 17	51 ± 8	46 ± 7	119 ± 9	100 ± 14	87 ± 9	86 ± 10	403 ± 25	398 ± 26
NH_3–N dep	not measured		0.39	0.39	0.68	0.68	1.31	1.31	2.38	2.38
NH_3–N em	not measured		0.38	0.55	0.43	0.55	0.34	0.41	1.15	1.51
Net NH_3–N ex			0.01	–0.16	0.25	0.13	0.97	0.90	1.23	0.87

mostly due to the volatilization of NH_3 from fertilizer particles, but some NH_3 emission from plant residues or newly growing leaves cannot be ruled out because uncontaminated samples of apoplast fluid could not be obtained immediately after fertilization. Likewise, emission of NH_3 originating from the soil cannot be distinguished either.

Integrating NH_3 emission and deposition over several regrowth periods reveals that, relative to the total N budget for above ground plant biomass, only a small amount of N was exchanged between the plant canopy and the atmosphere. Net NH_3 deposition calculated over three regrowth periods accounted for less than 1% of the total N yield, in spite of the fact that NH_3 fluxes occurring before the first cut were not included in the calculation because of missing NH_3 flux data for the early spring period. Similar NH_3 canopy compensation points for both N treatments indicate that the equal N budget of the two N treatments was associated with equal canopy sink strength for NH_3. NH_3 emission originating from fertilizer application was overcompensated by NH_3 deposition when integrated over the measuring period. In comparison, a net emission of NH_3 from cut and fertilized grasslands with mostly acidic soils was found under UK conditions, with a typical emission factor of 1.6% of N applied (van der Weerden and Jarvis, 1997).

NH_3 emission after fertilization would most probably have been higher with the application of organic fertilizers, such as slurry, which is more common practice in Switzerland than mineral N fertilization of grasslands. Depending on the application technique, losses of up to 70% of the NH_4–N applied with slurry have been reported (Dittert et al., 1998; Lockyer et al., 1989, Menzi et al., 1997). From the present study, we can conclude that with NH_4NO_3 fertilization at N-input levels commonly used for cut clover/grass systems, which is between 120 and 180 kg N ha^{-1} a^{-1}, net NH_3 emission is restricted to short periods after fertilizer application, and that for a grass/clover crop under field cutting conditions the source of N is irrelevant for the seasonal exchange of NH_3 between the canopy and the atmosphere.

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