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Temporal variability in bioassays of ammonia exchange potential in relation to plant and soil nitrogen parameters in intensively managed grassland

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Abstract

The exchange of ammonia between crop canopies and the atmosphere depends on a range of plant parameters and climatic conditions but little is known about effects of management factors. We have here investigated the ammonia exchange potential of a grass sward dominated by *Lolium perenne* in response to cutting and fertilization. Tall grass showed a low potential for NH_3 emission before cutting. During re-growth after cutting, leaf tissue concentrations of NO_3^-, NH_4^+, soluble N and total N increased along with apoplastic NH_4^+concentrations. In contrast, apoplastic pH decreased resulting in largely unaltered NH_3 emission potential. A high potential for NH_3 emission was shown by the plant litter. Fertilization with 100 kg N ha^{-1} one week after cutting caused the apoplastic NH_4^+ concentration of the newly emerging leaves to increase dramatically. The apoplastic NH_4^+ concentration peaked the day after the fertiliser was applied and thereafter decreased over the following 10 days until reaching the same level as before fertilisation. A positive correlation was found between NH_4^+ concentrations in leaf apoplast, bulk tissue and litter throughout the experimental period. Leaf soluble N was negatively correlated with apoplastic NH_4^+ concentration whereas total N was weakly correlated with NH_4^+ concentrations in leaf tissue and soil.

1 Introduction

Ammonia is emitted from plants when the atmospheric NH_3 concentration is lower than the NH_3 compensation point (i.e. NH_3 concentration in the substomatal cavity), while deposition of NH_3 occurs in the opposite situation (Farquhar et al., 1980; Husted et al., 1996). The NH_3 exchange potential of crops may change with management practise and climatic conditions (Sommer et al., 2004). Seasonal variations in climatic conditions affect NH_3 loss from crops (Schjoerring and Mattsson, 2001). In particular, temperature is known to have a major effect on the NH_3 exchange under controlled environmental conditions (Husted and Schjoerring, 1996; Mattsson et al., 1997) as

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well as in the field (van Hove et al., 2002; Trebs et al., 2006).

The NH_3 emission potential of grasslands may vary with species composition (Horvath et al., 2005; Trebs et al., 2006) because grass species differ in NH_3 compensation point as has been demonstrated both in cuvette measurements (Hanstein et al., 1999; Mattsson and Schjoerring, 2002) and in field studies (Herrmann et al., 2001; Mattsson et al., 2008). In a 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). Little is known about the NH_3 emission potential of grasslands where repeated cuttings and N fertilisations are normal management practice. Nitrogen fertilisation is one of the major management factors of grasslands and NH_3 volatilisation can be influenced by form, timing and dosage of N fertiliser (Riedo et al., 2002). Measurements of NH_3 volatilisation under controlled laboratory conditions have shown that high amounts of N supplied to the roots increase NH_3 emission (Mattsson et al., 1998, Mattson and Schjoerring, 1996) and NH_3 compensation points (Mattsson and Schjoerring, 2002). Increasing the N availability to plant roots leads to elevated steady state levels of different N pools within the plant tissue. In a field experiment over two years, the NH_3 losses from wheat, oilseed rape and barley increased under conditions of high N concentration in the foliage (Schjoerring and Mattsson, 2001). In a Scottish experiment, a higher NH_3 compensation point of the grass was seen after only one of the two cuttings and fertilisations (Loubet et al., 2002). A better understanding of the component parameters influencing the NH_3 emission potential is needed in order to model NH_3 exchange between grasslands and the atmosphere.

The aim of this study was to estimate the NH_3 emission potential of grassland in relation to common management practise. In order to do this, temporal variation in the NH_3 compensation point and its underlying components of grass leaves and soil was followed at a field site from before cutting to after cutting and lifting and subsequent N-fertilization of the field.

2 Materials and methods

The investigation took place as part of the GRAMINAE integrated experiment conducted on a field near Braunschweig from 22 May to 15 June 2000. The main field was 600×300 m in size and consisted of a mixed sward dominated by *Lolium perenne* as described in Mattsson et al. (2008). The grass was cut on 29 May and lifted for silage on 31 May. An area of 10×10 m was left uncut for additional sampling of tall grass. Fertilizer (100 kg N ha^{-1} in calcium ammonium nitrate) was applied on the main field on the 5 June. A 10×10 m plot was left unfertilized and another plot of the same size received 200 kg N ha^{-1} in calcium ammonium nitrate. Growth and development of the grass were as described in Sutton et al. (2008)[1].

2.1 Sampling of plant material

During the entire experiment plants were sampled almost every day between 12:00 and 3:00 p.m. (GMT). Cut green leaves were immediately taken to the field laboratory where the apoplastic solution was extracted using a vacuum infiltration technique (Husted and Schjoerring, 1995). Whole leaves were infiltrated in isotonic sorbitol solution (280 mM) at a pressure of 16 bar under vacuum for 5 s. The procedure was repeated 5 times in order to ensure full infiltration. Infiltrated leaves were carefully blotted dry and kept in plastic bags to equilibrate for 15 min in daylight. The leaf apoplast was extracted by centrifugation at 800 g for 10 min at 4°C. After extraction pH of the apoplastic samples was measured with a micro-combination pH electrode (9810, Orion, Beverly, USA) and samples were frozen at −18°C. Leaf samples for bulk tissue NH_4^+ and

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NO_3^- analysis were also frozen down at the same time for later extraction. Samples from litter (senescent leaves) and stubble (cut stems) were frozen down every day after the cut. For total N concentration, samples from leaves, litter and stubbles were taken daily and immediately dried in an oven (70°C) over night. Guttation droplets were collected on the main field and the high fertilized plot between 3:00 and 6:00 a.m. and immediately frozen.

2.2 Plant analysis

Ammonium in apoplastic extracts was determined by fluorometry on an HPLC system (Waters Corp. Milford, USA) equipped with a pump, a column oven with a 3.3 m stainless steel reaction coil, an autosampler cooled to 2°C and a scanning fluorescence detector. The reaction between NH_4^+ and o-phthaldehyde (OPA) to form an alkylthioisoindole fluorochrome was performed at neutral pH with 2-mercaptoethanol as reducing agent. This fluorochrome was detected at an exitation wavelength of 410 nm and an emission wavelength of 470 nm (Husted et al., 2000a).

The plant leaves, litter and stubble were homogenised in 10 mM formic acid in a cooled mortar with a little sand. The homogenate was centrifuged at 25 000 g (2°C) for 10 min and the supernatant was transferred to 500-μl 0.45 μm polysulphone centrifugation filters (Micro VectraSpin, Whatman Ltd, Maidstone, UK) and spun at 5000 g (2°C) for 5 min. 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.

2.3 Soil sampling and analysis

Soil samples were taken at least every third to fourth day with a soil auger at random positions over the field. Soil cores were separated into two layers (0–10 cm and 10–30 cm) and frozen at −18°C. A sub-sample was analysed for moisture content by calculating % weight loss after drying the soil for 24 h at 108°C. Another sub-sample (10 g) was used for pH measurements after extraction for 1 h in 25 ml 0.01 M $CaCl_2$. Plant available NH_4^+ and NO_3^- were analysed with flow injection after extraction of 25 g of soil in 50 ml 2 M KCl.

2.4 Calculation of NH_3 compensation point

The stomatal NH_3 compensation point (χ_s) at 25°C, $\chi_{s.25}$ (mol NH_3 mol^{-1} air) was calculated as:

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

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_s at 25°C was adjusted to the actual canopy temperature $t2$ in °C by the following equation derived from Husted and Schjoerring (1996):

$$\ln(\chi_{s.t2}/\chi_{s.25}) = (\Delta H^0_{dis} + \Delta H^0_{vap})/R \times (1/298.15 - 1/T_2) = 34.868 - 10395.91/(273.15 + t_2) \quad (2)$$

where $\chi_{s.t2}$ is the calculated NH_3 compensation point at the actual temperature t_2 (°C) ΔH^0_{dis} the enthalpy of NH_4^+ dissociation (52.21 kJ mol^{-1}), ΔH^0_{vap} the enthalpy of vaporization (34.18 kJ mol^{-1}), and R the gas constant (8.31 J K^{-1} mol).

3 Results

3.1 Apoplastic parameters

Data presented here for the tall grass leaves are averages of the mean values of the 3 most abundant species (*Lolium perenne, Phleum pratense and Festuca pratensis*), weighted by their relative abundance in the grass field. The apoplastic NH_4^+ concentration was below 50 μM in the tall grass both before and after the main field was cut (Fig. 1a). New leaves of main field developing after cutting, i.e. during the period of regrowth, showed slightly increased apoplastic NH_4^+ concentrations compared to leaves of the tall grass. Following application of 100 kg N ha^{-1} to the main field 6 days after cutting of the grass, apoplastic NH_4^+ concentrations rapidly peaked but thereafter decreased during the next 10 days until almost the same level was reached as before fertilisation (Fig. 1a). Plants on a plot receiving 200 kg N ha^{-1} did not show higher apoplastic NH_4^+ concentrations compared to the main field (Fig. 1a). When no nitrogen was applied (0 N plot) apoplastic NH_4^+ concentrations remained below 100 μM throughout the experimental period. Apoplastic pH was higher in the tall grass compared to the cut grass (Fig. 1b). Fertilisation caused a transient increase in apoplastic pH without showing any difference between plants receiving 100 or 200 kg N ha^{-1} (Fig. 1b). Values of Γ (dimensionless, temperature independent ratio between apoplastic NH_4^+ and H^+ concentrations) showed a similar pattern as the apoplastic NH_4^+ concentrations with very low values of 10 to 150 before fertilisation, above 1000 the first 2 days after fertilisation and thereafter slowly decreasing values (Fig. 1c). In the 0 N plot, Γ remained below 150 during the whole experiment. The slight increase in apoplastic NH_4^+ concentrations following cutting was counteracted by decreasing pH values therefore Γ values hardly changed between cutting and fertilisation. The calculated stomatal NH_3 compensation point χ_s corrected for temperature differences between different days showed values of 1–2 nmol mol^{-1} before cutting and for unfertilised grass after cutting (data not shown). After N fertilisation, χ_s peaked at 15–25 nmol mol^{-1} but already 4

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days after fertilisation it had decreased to 3–4 nmol mol^{-1} (not shown).

3.2 Tissue parameters

Bulk tissue NH_4^+ concentrations of the tall grass as well as the cut grass prior to fertilisation were lower than 2 μmol g^{-1} FW (Fig. 2a). After fertilisation, bulk tissue NH_4^+ concentrations increased rapidly and substantially, peaking around 14 μmol g^{-1} FW with little difference between 100 and 200 kg N ha^{-1} treatments (Fig. 2a). Plants not receiving N fertilizer (0 N treatment) maintained a bulk tissue NH_4^+ level below 4 μmol g^{-1} FW.

Bulk tissue NO_3^- concentrations were extremely low in the tall grass (Fig. 2b), while in the new leaves developing after the cut, the NO_3^- concentration increased considerably. Fertilisation caused a dramatic increase (4 to 5 fold) in bulk tissue NO_3^- concentrations and the high level of NO_3^- remained until the end of the experiment (Fig. 2b). In unfertilised grass, NO_3^- concentrations decreased towards the end of the experiment to values similar to those of the tall grass.

Total N concentration in the tall grass leaves decreased from 3% (dry weight basis) before the cut to about 2% 9 days later (Fig. 3b). The remaining part of the cut stems (stubbles) also had a total N concentration of ca. 2% throughout the rest of the experiment. In the newly produced leaves of the 100 N treatment (main field), the total N concentration increased from around 3% just after cutting to around 5% at the end of the experiment (Fig. 3b). The final foliar N concentration in the 0 and 200 N treatments were 3.5% and 5.5%, respectively (not shown).

Tissue extracts were also analysed for total soluble N concentration which can be interpreted as a dynamic N pool available for plant growth. This so-called "substrate N" was very high in leaves remaining or developing after cutting (Fig. 3a). Following fertilisation, plants in the 100 and 200 N treatments had significantly higher substrate N than unfertilised grass (0 N treatment). Substrate N constituted between 10 and 40% of total leaf N.

The litter component of the grassland consisting of senescent plant leaves either attached to the lower part of the stems or lying on the ground constituted about 20% of the total above-ground biomass before cutting (data not shown). Prior to cutting, litter concentrations of NH_4^+ and NO_3^- were below 50 mM, while 3 days after cutting, the NO_3^- concentration in the litter had increased from below 50 mM to about 350 mM, while litter NH_4^+ remained below 50 mM (Fig. 4). After fertilization (100 N), litter concentrations of NH_4^+ increased to around 500 mM but already after a few days they started to decrease again (Fig. 4). Nitrate concentrations were slightly higher than NH_4^+ concentrations after fertilisation but followed the same temporal pattern (Fig. 4).

In parallel to apoplastic extracts, NH_4^+ and H^+ concentrations in bulk tissue extracts can be used to derive Γ values for different plant fractions of the sward in order to derive their potential NH_3 exchange (Table 1). Bulk pH values of green leaves and stems were similar to those in the apoplastic solution ranging between 6.2 and 6.6 (Table 1; Fig. 1b). Due to higher NH_4^+ concentrations in the tissue extracts (Table 1) than in the apoplastic solution (Fig. 1a), the resulting Γ value in green leaves were relatively high (around 300; Table 1) compared to Γ values around or below 100 in the corresponding apoplastic solution (Fig. 1c). Extracts of plant litter showed both higher pH and, particularly, NH_4^+ concentrations compared to leaves and stems which resulted in a Γ values higher than 5000 (Table 1).

3.3 Soil parameters

Soil NH_4^+ and NO_3^- concentrations (Fig. 5) were low before fertilisation with NH_4^+ concentrations being higher than NO_3^- concentrations in both the top soil fraction (0–10 cm) and the lower soil fraction (10–30 cm, not shown). The top soil concentrations of both NH_4^+ and NO_3^- increased dramatically after application of 100 kg N ha^{-1}, while soil inorganic N increased less in the 200 N treatment. The N fertiliser in the 200 N plot was applied by hand a few hours later and under drier conditions compared to that in the 100 N treatment of the main field where rainfall followed within a couple of hours after the application.

3.4 Correlation analysis

A correlation analysis of all the obtained results showed that apoplastic pH and the NH_4^+ concentration in guttation droplets were not related to any of the other parameters (Table 2). The NH_4^+ concentrations in apoplastic solution, leaf tissue and litter were mutually positively correlated. Leaf tissue and litter NH_4^+ concentrations were also positively correlated with the NH_3 compensation point derived from the apoplastic measurements (Table 2). Substrate N (Fig. 3) was negatively correlated with apoplastic NH_4^+ concentration, while total leaf N on a dry weight basis was positively correlated with NH_4^+ in leaf tissue and soil.

4 Discussion

Before cutting, the tall grass had low NH_4^+ concentrations in both apoplast and leaf tissue (Fig. 1a, 2a). This resulted in NH_3 compensation points so low that the grass was not likely to emit NH_3 before cutting which is in agreement with NH_3 gradients above the canopy showing predominantly deposition fluxes (Milford et al., 2008[2]). Extremely low tissue NO_3^- concentrations (Fig. 2b) and low soil NH_4^+ and NO_3^- concentrations (Fig. 5) also indicated that the small amounts of inorganic N available to the plants were efficiently taken up and utilised for growth and seed development at this stage. Before fertilisation there was 4 times higher concentrations of NH_4^+ than NO_3^- in the soil (Fig. 5), which is not unusual for grassland soil (Whitehead, 1995).

Between cutting and fertilisation there was a re-growth period of a week when the grass first showed increased NO_3^- concentrations (Fig. 2b) and 5 days after the cut also increased NH_4^+ concentration in the leaf tissue (Fig. 2a). Ryegrass has been shown to

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rapidly accumulate NO_3^- in both leaves and stubble after cutting as NO_3^- is involved in the osmotic adjustment (Ourry et al., 1989). Also the soluble N and total N concentrations of the leaves showed higher values during re-growth after cutting compared to the tall grass (Fig. 3a, b). These increased N pools in the grass leaves seemed to be correlated with slightly increasing NO_3^- and NH_4^+ concentrations in the soil. Several authors have reported that during the first days after cutting, uptake of N is inhibited (Bakken et al., 1998; Ourry et al., 1988). In such case, plants respond by allocating N from reserves in root and stubble to the developing leaves. This inhibition of uptake could explain the increasing levels of soil NO_3^- and NH_4^+ after the cut. In *Lolium perenne* and *Bromus erectus* grown in nutrient solution, the tissue NH_4^+ concentrations of expanding leaves did not start to increase until 6 days after the cut (Sutton et al., 2001). In the same experiment, apoplastic NH_4^+ concentrations increased in the new expanding leaves 3–6 days after cutting. Also in the present study, apoplastic NH_4^+ concentrations showed slightly higher values in expanding leaves during re-growth compared to the leaves of the tall grass (Fig. 1a). Increasing tissue concentrations of NH_4^+ and NO_3^- can also result from shortening of the leaf growth zone and smaller dilution of the N transported to this zone after defoliation compared to the fully expanded leaves before cutting (Schäufele and Schnyder, 2001).

Ammonia is typically emitted from grassland rather than deposited following cutting as demonstrated by micro-meterological measurements (Milford et al., 1999, 2002; Loubet et al., 2001). Such NH_3 emission could originate from the plants as a consequence of increased N pools during the period of leaf expansion. However, the potential for NH_3 emission did not seem to increase since the Γ values (the ratio between the NH_4^+ and H^+ concentration) were unaltered due to counteracting effects of the decreased apoplastic pH and the increased apoplastic NH_4^+ concentrations (Fig. 1c). Another source of NH_3 emission could be the litter, i.e. senescent leaves attached to the stems or lying on the ground surface. Ammonium concentrations were considerably higher in the litter material compared to green leaves (Fig. 4) due to the breakdown processes going on in this material (Mattsson and Schjoerring, 2003). These

senescence-related processes were probably also enhanced after cutting when both the climatic conditions and the proportion of litter out of total biomass were changed at the bottom of the canopy (David et al., 2008[3]). In a tall canopy, NH_3 emitted from the litter can be taken up by leaves positioned higher above the ground (Husted et al., 2000b; Nemitz et al., 2000), while in the absence of a tall canopy the litter NH_3 will escape to the atmosphere. High NH_4^+ concentrations and relatively high pH values in litter material also resulted in Γ values of above 5000, which is a clear indication of NH_3 emission (Table 1). In a non-fertilized grassland in the Netherlands, Wichink Kruit et al. (2007) observed an average canopy Γ value of 2200.

After fertilisation, all plant N pools increased with peak values already on the first day after fertilisation. Micrometeorological measurements also showed high NH_3 emissions after fertilisation with some contribution from the fertiliser itself during the first 2 days (Milford et al., 2008[2]). The fertiliser was rapidly dissolved in the soil solution since it was raining the same afternoon as the main field was fertilised (Sutton et al., 2008[1]) and therefore the fertiliser contamination was restricted to a very short period. Both NO_3^- and NH_4^+ were taken up from the soil since leaf tissue concentrations increased dramatically (Fig. 2). The fertilisation was also reflected in higher NH_4^+ concentrations in guttation droplets collected at the leaf tips in the early mornings after fertilisation (not shown).

Ammonium concentrations in both leaf tissue and apoplast starting decreasing again already a few days after fertilisation (Figs. 1a, 2a) while leaf tissue NO_3^- concentrations remained high for the rest of the experiment (Fig. 2b). This may partly reflect declining soil NH_4^+ levels (Fig. 5) and partly rapid assimilation of NH_4^+ in the plant cells, while NO_3^- was stored in the leaf cell vacuoles for later use. It has previously been shown that when NH_4NO_3 is supplied to plant roots, NH_4^+ is absorbed more readily than NO_3^- (Bloom, 1981; Clarkson et al., 1986). Nitrate accumulates in grass herbage when the

[3]David, M., Loubet, B., Cellier, P., Mattsson, M., Schjoerring, J. K., Nemitz, E., Roche, R., Riedo, M., and Sutton, M. A.: Ammonia sources and sinks in an intensively managed grassland using dynamic chambers, Biogeosciences Discuss., to be submitted, 2008.

rate of uptake by the roots exceeds the rate of conversion to organic N (Whitehead, 1995). The NH_4^+ concentrations in apoplast and bulk tissue were obviously sensitive parameters responding rapidly to fluctuations in soil nitrogen availability. Also in a laboratory experiment with *Lolium perenne* and *Bromus erectus* both leaf tissue and apoplastic NH_4^+ concentrations were shown to respond rapidly to changing NH_4^+ concentrations in the nutrient solution (Mattsson and Schjoerring, 2002).

A correlation analysis revealed that NH_4^+ concentrations in leaves and litter were positively correlated with the stomatal NH_3 compensation point calculated on the basis of apoplastic parameters (Table 2). Some previous investigations have similarly shown good correlation between apoplastic and leaf tissue NH_4^+ concentrations (Mattsson et al., 1998; Mattsson and Schjoerring, 2002), suggesting that the tissue NH_4^+ concentration may be used as an indicator of the NH_3 compensation point. Other studies have found the correlation to depend on growth conditions (Herrmann et al., 2008) or not to be present (Hill et al., 2002).

5 Conclusions

We conclude that the management practice has a major influence on the the potential plant-atmosphere exchange in grassland by influencing both plant and soil N parameters. NH_4^+ concentrations in bulk leaf tissue and litter were positively correlated with the NH_3 compensation point derived from apoplastic measurements. This suggests that measurements of NH_4^+ and pH in bulk extracts of plant material in grassland can be used as a simple indicator of the NH_3 exchange potential.

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Table 1. Tissue extracts of green leaves, stems and senescent leaves (litter) of the main field analysed for pH, NH_4^+ concentration and the ratio Γ between $[NH_4^+]$ and $[H^+]$. Means of 3 replicates ±SE.

	pH	$[NH_4^+]$, mM	Γ
Green leaves	6.33±0.02	1.79±0.01	305±1.5
Stems	6.37±0.04	1.15±0.1	190±21
Senescent leaves	7.03±0.05	16.2±1.2	5193±392

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Table 2. Correlation coefficient table for all the different parameters measured and calculated in grass plants and soil from the 100 N treatment (main field). r^2 values with level of significance (*=0.05; **=0.01, ***=0.001).

	$[NH_4^+]_{apo}$	pH_{apo}	$[NH_4^+]_{tissue}$	χ_{NH_3}	$[NH_4^+]_{litter}$	$[NH_4^+]_{guttation}$	SubstN	TotN	$[NH_4^+]_{soil}$
$[NH_4^+]_{apo}$	–	0.08	0.60***	0.84***	0.50***	0.22	0.48***	0.27	0.23
pH_{apo}	0.08	–	0.02	0.21	0.07	0.02	0.16	0.15	0.004
$[NH_4^+]_{tissue}$	0.60***	0.02	–	0.48***	0.73***	0	0.35**	0.42**	0.29
χ_{NH_3}	0.84***	0.21	0.48***	–	0.46***	0.01	0.25*	0.15	0.21
$[NH_4^+]_{litter}$	0.50***	0.07	0.73***	0.46***	–	0.34	0.40	0.21	0.47*
$[NH_4^+]_{guttation}$	0.22	0.02	0	0.01	0.34	–	0.19	0.06	0.19
SubstN	0.48***	0.16	0.35**	0.25*	0.40	0.19	–	0.23	0.44
TotN	0.27	0.15	0.42**	0.15	0.21	0.06	0.23	–	0.68*
$[NH_4^+]_{soil}$	0.23	0.004	0.29	0.21	0.47*	0.19	0.44	0.68*	–

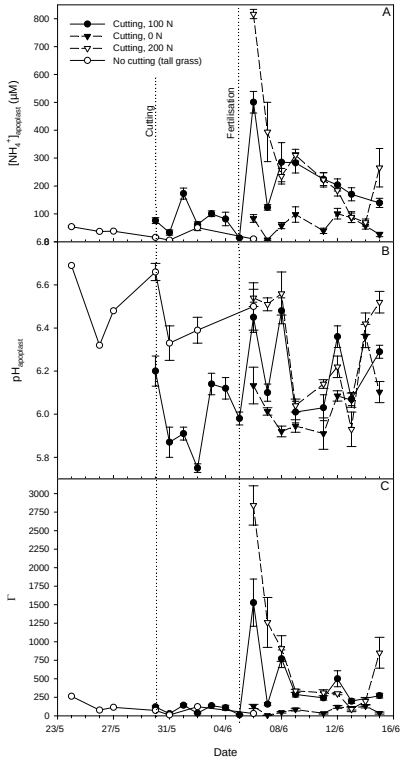

Fig. 1. Temporal variation in **(A)** apoplastic $[NH_4^+]$, **(B)** apoplastic pH, and **(C)** the ratio of $[NH_4^+]_{apoplast}$ to $[H^+]_{apoplast}$ (Γ) in four treatments of a *Lolium perenne* dominated sward near Braunschweig (Germany) in 2000. The grass was cut on 29 May and lifted for silage on 31 May. An area of 100 m^2 was left uncut for additional sampling of tall grass. Fertilizer (100 kg N ha^{-1} in calcium ammonium nitrate) was applied on the main field on 5 June. A 100 m^2 plot was left unfertilized and another plot of same size was applied 200 kg N ha^{-1} in calcium ammonium nitrate. Vertical dotted lines indicate times of cutting and fertilisation, respectively. Values are means of three replicates ±S.E.

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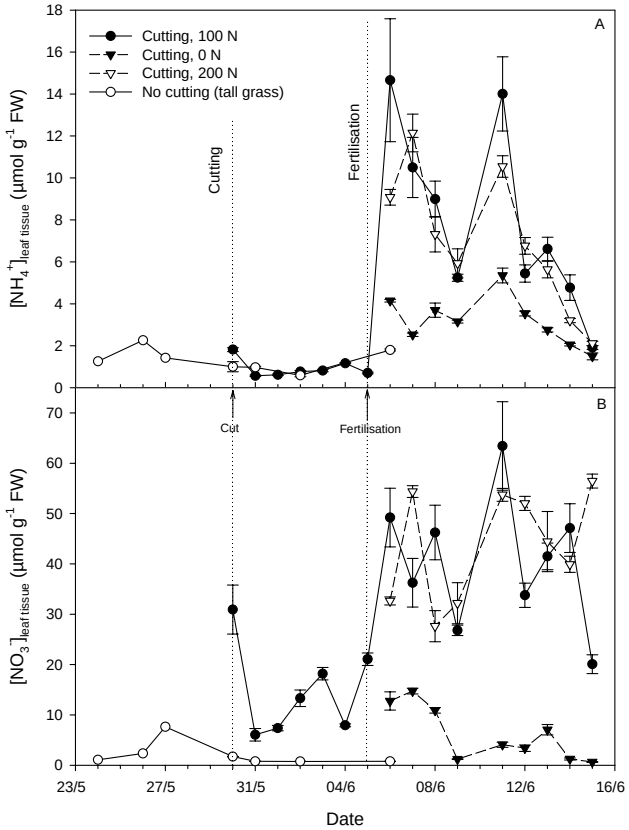

Fig. 2. Temporal variation in **(A)** leaf $[NH_4^+]$ and **(B)** leaf $[NO_3^-]$ on a fresh weight basis in four treatments of a *Lolium perenne* dominated sward near Braunschweig (Germany) in 2000. Details on experimental treatments are given in Fig. 1. Values represent means of three replicates ±S.E.

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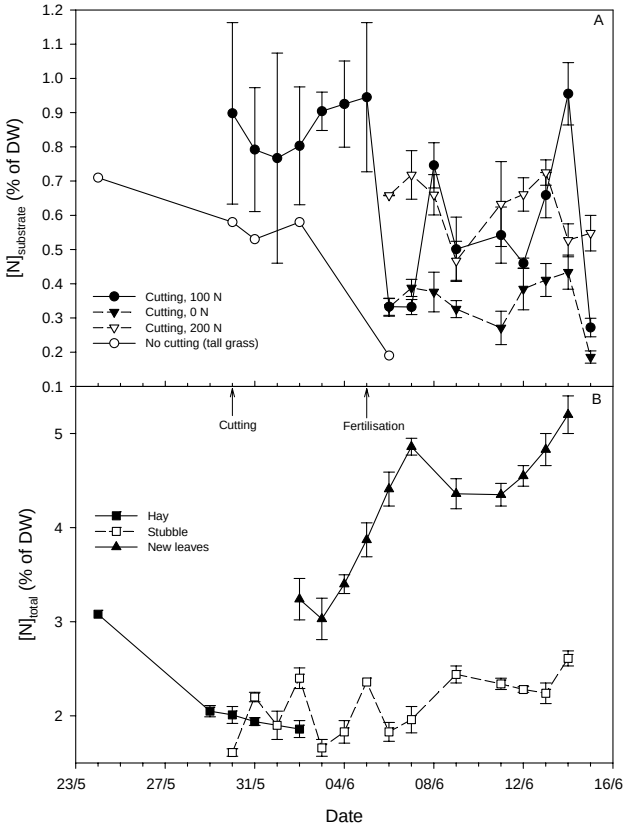

Fig. 3. Temporal variation in **(A)** "substrate [N]" on dry weight basis of leaf tissue in four treatments of a *Lolium perenne* dominated sward near Braunschweig (Germany) in 2000 and **(B)** total [N] on a dry weight basis of three components of the tall grass treatment. $[N]_{substrate}$ denotes the concentration of total soluble N available for growth. Details on experimental treatments are given in Fig. 1. Arrows indicate times of cutting and fertilisation, respectively. Values represent means of three replicates ±S.E.

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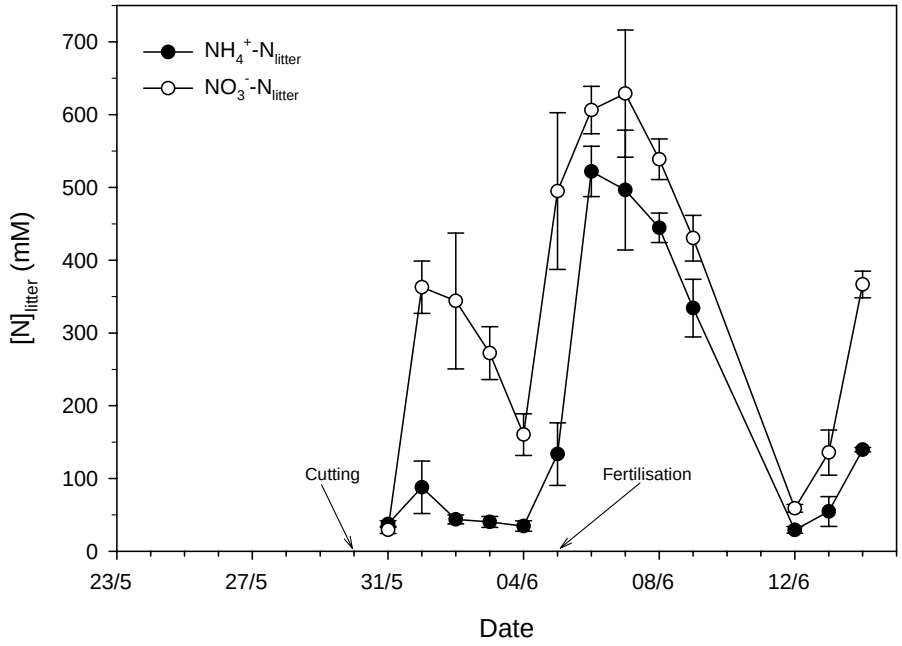

Fig. 4. Temporal variation in $[NH_4^+]$ and $[NO_3^-]$ of the litter lifted a *Lolium perenne* dominated sward near Braunschweig (Germany) in 2000. Details on experimental treatments are given in Fig. 1. Arrows indicate times of cutting and fertilisation, respectively. Values represent means of three replicates ±S.E.

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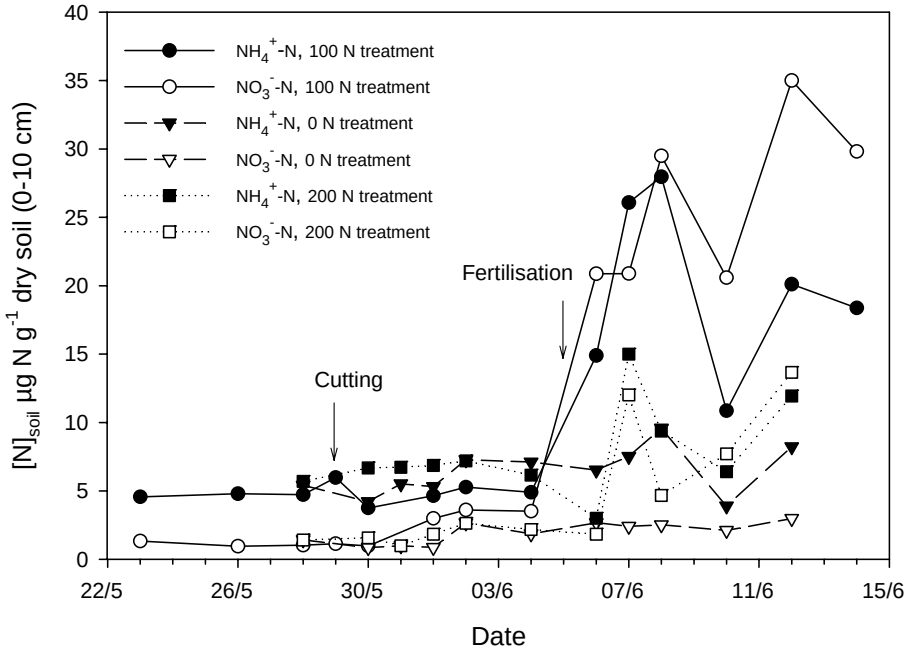

Fig. 5. Temporal variation in $[NH_4^+]$ and $[NO_3^-]$ of the top layer of soil in three treatments of a *Lolium perenne* dominated sward near Braunschweig (Germany) in 2000. Details on experimental treatments are given in Fig. 1. Arrows indicate times of cutting and fertilisation, respectively.

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