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Published version:

DOI:10.1111/ejss.12391

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Cavalli, D.; Marino Gallina, P; Sacco, D.; Bechini, L.. Soil mineral nitrogen dynamics following repeated application of dairy slurry. EUROPEAN JOURNAL OF SOIL SCIENCE. 67 (6) pp: 804-815.
DOI: 10.1111/ejss.12391

Soil mineral nitrogen dynamics following repeated application of dairy slurry

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Running title: Soil nitrogen dynamics after dairy slurry application

Keywords: Animal manure, nitrogen residual effect, nitrogen recovery, non-exchangeable ammonium, clay fixation

Research highlights:

- **A novel incubation approach** was used to s**tudy residual N effects of ammonium sulphate and slurries**
- **Fertilizers were applied one, two, three or four times to a sandy loam (SL) and a clay loam (CL) soil**
- **Residual N effects were small; less slurry NH_4-N was available in CL than SL because of clay fixation**

- **Mineralization of residual slurry-N and stabilization of microbial by-products were slow**

Summary

Repeated applications of animal slurry to soil can lead to residual nitrogen (N) effects from mineralization of organic N carried over from the previous year and from re-mineralization of previously immobilized N. We studied the effect of repeated slurry applications on soil mineral N (SMN_t: nitrate-N plus soluble, exchangeable and non-exchangeable ammonium-N) dynamics in a simplified, aerobic laboratory incubation. The experiment evaluated the effects of up to four applications (84-day intervals) of two different liquid cow slurries, ammonium sulphate and water (unfertilized control, CON) to sandy loam and clay loam soils. The slurries came from heifers (HEI) and lactating dairy cows (COW). Both soil types showed net N mineralization in HEI during each 84-day interval after application (3–6% of slurry-N), whereas decomposition of COW induced net N immobilization at 16% of slurry-N. The effect observed for COW might have come from its larger C to organic-N ratio. After each application to the clay loam soil, 36% to 64% of the ammonium applied was not recoverable at Day 0 because of ammonium fixation by clay minerals, and an average of 20% of fertilizer-N was measured as non-exchangeable ammonium at Day 84. Recovery of N applied with both HEI and COW at Day 84 increased significantly with subsequent applications to clay loam soil, but not to sandy loam soil. Residual effects in clay loam soil ranged from 2 to 11% of applied N, which probably resulted from slow mineralization of recalcitrant organic fractions in the slurry and partial stabilization of microbial by-products within the soil.

Introduction

Fertilization of soil with animal manures can extend nitrogen (N) availability to crops beyond the year of application because mineralization of organic N can carry on from one season to the next (Webb *et al.*, 2013). Livestock farmers typically apply manure to the same land every year, and a residual effect can emerge within a few years (Schröder *et al.*, 2005 and 2007). This residual N then gives rise to larger rates of N mineralization (Whalen *et al.*, 2001) and nitrification (Luxhøi *et al.*, 2004) in soil treated continuously with manure than in soil where manure is applied only occasionally or never.

Several factors affect the soil residual effect. Soil type is likely to be one because texture influences the rate of decomposition of added organic matter (OM) (Six *et al.*, 2002). Previous evidence (Thomsen & Olesen, 2000; Thomsen *et al.*, 2003) leads us to hypothesize that the residual N effect will be greater on finer- than coarser-textured soil because of slower and more prolonged decomposition of OM in clayey than sandy soil. This is because of the physicochemical protection of added organic matter and microbial by-products by clays.

Soil mineral composition has also been shown to have a strong effect on mineral N availability for microorganisms through ammonium clay fixation in non-exchangeable form (Nõmmik & Vahtras, 1982; Nieder *et al.*, 2011), which indirectly affects organic matter turnover. The mechanism of ammonium fixation includes the sorption of NH_4^+ ions (similar to K^+, Cs^+ and Rb^+) into the interlayers of 2:1 type clay minerals, and successive collapse (a reduction of the basal spacing) of the crystal lattice until fixed ions are almost excluded from exchange reactions for weeks or months (Nõmmik & Vahtras, 1982). Sites of NH_4^+ fixation were identified at the frayed edges of illite (weathered mica) and interlayer positions of expandable clay minerals such as vermiculite, and to a lesser extent some smectites (Sawhney, 1972; Nõmmik & Vahtras, 1982). Ammonium fixation usually occurs quickly (within hours), whereas its release takes more time (weeks or even months), therefore, fixed (non-exchangeable) NH_4^+ ions become available slowly to soil microorganisms (Nõmmik & Vahtras, 1982).

Finally, manure type might also affect the residual effect (Webb *et al.*, 2013). Specifically, manures with slower rates of decomposition leave larger amounts of undecomposed residual organic N after the year of application, which results in a more pronounced residual effect in subsequent years (Webb *et al.*, 2013).

Residual effects are traditionally assessed in field experiments (Cusick *et al.* 2006; Schröder *et al.*, 2007; Monaco *et al.*, 2010; Cavalli *et al.*, 2016); however, it is difficult to do so accurately because of soil spatial variability and measurement uncertainty of N loss. Moreover, the field is a difficult setting in which to conduct the type of soil and manure comparisons required in a factorial design. Aerobic laboratory incubations of manure-amended soil provide an alternative way in which to study the decomposition dynamics of manure (Bechini & Marino, 2009). The laboratory eliminates issues of nitrate leaching, crop uptake of N and effects of the crop on soil organic matter mineralization. Furthermore, experimental conditions can be controlled (immediate and accurate soil–manure mixing, constant soil water content and temperature) to standardize the study of organic matter turnover.

Clearly, laboratory studies do not fully mirror a real system, but they are effective for comparing the dynamics of mineralization with different types of soil and manures, and for measuring net N mineralization. Although already used in research on the effects of manure composition on C and N mineralization after a single application on one or more soil types (Kirchmann & Lundvall, 1993; Sørensen & Jensen, 1995; Sørensen, 1998; Morvan *et al.*, 2006), to our knowledge the controlled conditions of a laboratory incubation study have yet to be used to develop our understanding of the residual effects of different manures. We consider that our approach is both novel and promising. We designed, conducted and reported (Cavalli *et al.*, 2014) the effects of C respiration in an aerobic laboratory incubation that considered four additions of two different cow slurries on two soil types of different texture. Here, we report the partitioning of mineral N into different fractions (nitrate, exchangeable ammonium and non-exchangeable ammonium), and quantify the residual N effect of the different slurries and soil types considered.

Materials and methods

Treatments and experimental set-up

The incubation experiment considered a full combination of the following factors: soil type (two levels), fertilizer type (four levels) and number of cumulated fertilizer applications (four levels). There were 32 treatment combinations in total.

Table 1 summarizes the physicochemical characteristics of the sandy loam and clay loam soil used in the laboratory incubation. They differed principally in clay and sand content (40 and 666 g kg^{-1}, respectively, in the sandy loam soil, 305 and 448 g kg^{-1}, respectively, in the clay loam soil), but they were alike in that both had received no organic fertilizers during the decade preceding the sample collection (summer 2009) and both had a neutral pH in water. Before the start of the experiment, both soils were air-dried and sieved to pass through a 2-mm mesh. Thereafter, they were remoistened and incubated at 25°C for one week to reactivate their microbial biomasses and to mineralize most of their remaining labile organic matter.

In addition to soil type, the experiment considered fertilizer type and number of applications as variables. The fertilizers included two slurries, an unfertilized control (CON) and a mineral fertilizer control (ammonium sulphate, AS). The characteristics of the slurries from heifers (HEI) and lactating dairy cows (COW) are given in Table 2. The cumulated fertilizer applications ranged from one to four (Applications 1, 2, 3 and 4), with an elapsed time of 84 days between any two applications (Figure 1).

After the final application of fertilizer type associated with each experimental unit, at Application 1, 2, 3 or 4, we measured soil N and pH on six dates during the 84-day interval at: 0, 1, 15, 29, 41 and 84 days (Figure 1). Sampling at Day 0 refers to two hours after fertilizer application. The experiment was arranged in a completely randomized design with three replicates. Destructive measurements were done on different experimental units on each date following Thuriès *et al.* (2000). Therefore, we prepared 576 experimental units (32 treatments × 6 dates × 3 replicates) for which measurements were done only once.

Each experimental unit consisted of pre-incubated soil (100 g dry weight) amended with water or one of the fertilizers, applied at 100 mg N kg^{-1} of dry soil. Each experimental unit underwent incubation in the dark at 25°C, and soil humidity (WC_{-50kPa}, Table 1) was kept constant by periodic additions of distilled water to compensate for evaporation. To avoid excessive soil water content from fertilizer application, all experimental units belonging to Applications 2, 3 and 4 were partially air-dried for three days before subsequent applications.

Measurement of pH and mineral nitrogen concentration

On all sampling dates, we measured the exchangeable ammonium concentration and nitrate concentration of the soil to estimate net slurry-N mineralization during incubation. At Day 84 after each application we also measured the non-exchangeable ammonium concentration in the clay loam soil so that this form of ammonium could be included in calculations of net slurry-N mineralization. We also measured soil pH on all sampling dates to give further support to the interpretation of mineral nitrogen dynamics.

Soluble and exchangeable NH_4-N and NO_3-N were extracted for 2 hours with a solution of 1M KCl (extraction ratio 1:3). The suspension was filtered through Whatman No 2 filter paper (Whatman International Ltd, Maidstone, England) and stored at –20°C until analysis (UNICHIM method 780:88; UNICHIM, 1988). Ammonium-N (NH_4-N) and nitrate-N (NO_3-N) concentrations in the soil extracts were determined by flow injection analysis and detected with a spectrometer (FIAstar 5000 Analyzer, Foss Tecator, Hillerød, Denmark). Analysis of NH_4-N was done by the gas semi-permeable membrane method of the ISO 11732 procedure (1997). We used the sulphanilamide-naphtylethylendiamine dihydrochloride method to analyse NO_3-N after preliminary reduction of nitrate to nitrite with a copper–cadmium reduction column following the ISO 13395 procedure (1996).

Non-exchangeable NH_4-N was determined by the slightly modified method of Silva & Bremner (1966). Soil samples were oven-dried (25°C max) and ground by hand to pass through a 1-mm sieve (Beuters & Scherer, 2012), after which they were treated with an alkaline potassium hypobromite solution to remove exchangeable ammonium and organic N. Soil residues from this pretreatment were washed three times with 0.5M KCl and shaken for 24 hours with an acid solution (5M HF:1M HCl) to decompose any silicates. The ammonium concentration in the acid extract was then determined by steam distillation and titration. Distillation was done with a Büchi K-350 distillation unit (BÜCHI Labortechnik AG, Flawil, Switzerland) after preliminary alkalization of the extracts with a 32% NaOH solution (Beuters & Scherer, 2012). The distillate, collected in a beaker containing a 4% H_3BO_3 (boric acid) solution, was titrated with 0.005 M HCl using a G20 Compaq Titrator (Mettler-Toledo, Greifensee, Switzerland).

Soil pH was determined potentiometrically with a Crison GLP 21 + pH-meter (Crison S.A., Alella, Spain) on a soil–water mixture with a ratio of 1:2.5.

Calculations and statistical analysis

Soil exchangeable plus soluble mineral nitrogen (SMN_{es}) was calculated separately for each incubation period as the sum of NO_3-N and exchangeable NH_4-N. Total SMN (SMN_t) on the clay loam soil was calculated at the end of each application period (Day 84) as SMN_{es} plus non-exchangeable NH_4-N, whereas on the sandy loam soil SMN_t was assumed equal to SMN_{es} because of the lack of soil ammonium fixation by clay, which was confirmed by preliminary testing of the soil.

For each application event, net nitrogen recovery was calculated separately for exchangeable NH_4-N, NO_3-N, non-exchangeable NH_4-N (for clay loam soil only) and SMN_t; the recoveries were calculated as the differences between values measured in fertilized treatments and those measured in CON. Any resulting recoveries were expressed as a fraction of total applied N.

Nitrogen residual effects (NRE) on Day 84 of one, two or three fertilizer applications were calculated separately for each fertilizer type. Three estimates of NRE from one fertilizer application (NRE_1) were obtained from differences in recovery of SMN_t

between Applications 2 and 1, Applications 3 and 2, and Applications 4 and 3. Similarly, estimates of NRE after two fertilizer applications (NRE_2) were calculated as the difference in recovered SMN_t between Applications 3 and 1, and between Applications 4 and 2. There was one possible estimate only of NRE from three fertilizer applications (NRE_3); it was calculated as the difference between SMN_t recovery for Applications 4 and 1. Finally, each variance of NRE was determined from the sum of the two SMN_t recovery variances used in the estimate.

The statistical effects of soil type (SOIL), fertilizer type (FER), number of applications (APP), sampling date (DAY) and their interactions on soil pH or SMN_{es} (mg N kg^{-1}) were determined with a three-way ANOVA model that considered the following components: fixed factors SOIL, FER, APP and DAY; two-way interactions between all fixed factor pairs; three-way interactions SOIL × FER × APP, SOIL × APP × DAY and FER × APP × DAY. Means were compared with planned orthogonal contrasts. A set of polynomial contrasts was defined first to test for linear trend in soil pH or SMN_{es} during the 84 days within each FER × APP combination. We also expected soil pH to decrease during incubation, and SMN_{es} to accumulate in soil with additional applications. Therefore, a second set of polynomial contrasts was used to test for linear trend in soil pH or SMN_{es} from Application 1 to Application 4 within SOIL × FER combinations. Finally, to test our assumption that soil texture affected soil pH and mineral N concentration, a third set of contrasts was defined to assess the effect of SOIL on soil pH or SMN_{es}. The effect of SOIL was tested for each APP on three particular sampling dates: incubation period start (Day 0), short-term N immobilization finish (Day 15) and incubation period finish (Day 84).

A second three-way ANOVA was done to test the effect of SOIL, FER and APP on SMN_t (% applied N) on Day 84 with a full factorial model. The linear trend in SMN_t across applications was tested for each SOIL × FER combination by orthogonal polynomial contrasts.

A third two-way, full factorial ANOVA model was formulated to test the effects of FER and APP on Day 84 net non-exchangeable NH_4-N concentration (% applied N). For this model, we also defined orthogonal polynomial contrasts to identify any linear trend in net non-exchangeable NH_4-N concentration across applications separately for each FER.

All analyses of variance were carried out with the GLM procedure of SPSS, Version 22.0.0 (IBM Inc., Armonk, New York); contrasts were determined with the LMATRIX command. Significant differences in the means are reported when the *P* value was below 0.05.

Results

Soil pH

Soil pH measured during incubation (Figure 2) was affected significantly by interactions between soil type, fertilizer type, number of applications and sampling date (Supplementary Information, Table S1). Soil pH decreased significantly and often markedly over the period Day 0–84 for most of the treatments (from –0.11 to –0.69 pH units) (Supplementary Information, Table S2). The decline was larger and faster during Application 1 (from –0.13 to –0.69 pH units) than in subsequent applications (from –0.02 to –0.37 pH units).

In addition, soil pH showed a significant net decrease across applications (Supplementary Information, Table S3) in all treatments (from –0.12 to –1.74 pH units), except for HEI for both soil types (+0.37 and +0.73 pH units on sandy loam and clay loam soil, respectively). Soil pH was reduced substantially in AS to such an extent that after Application 1, pH had already fallen to 4.6 in sandy loam and 6.0 in clay loam soil, respectively. Further decline to 4.3 (sandy loam soil) and 4.4 (clay loam soil), occurred after Application 4. In spite of similar pH values before the start of the experiment (6.7 and 6.8 in the sandy loam and clay loam soil, respectively), the sandy loam soil always had a lower pH than the clay loam soil (from –1.26 to –0.62 pH units) (Supplementary Information, Table S4).

Soil mineral N dynamics

The ANOVA results (Table 3) showed that SMN_{es} was affected significantly by the interactions between soil type, fertilizer type, number of applications and sampling date. Table 4 lists the fitted increase in SMN_{es} over the 84 days during the four application periods. The fitted trend in SMN_{es} across the four applications is given in Supplementary Information (Table S5). In the CON treatment, exchangeable NH_4-N remained small (<5 mg N kg^{-1}) during the entire incubation period (data not shown). Over time, SMN_{es} increased significantly (Table 4) in CON (Figure 3a,b) because NO_3-N accumulated between Days 0 and 84 after each water application event. Moreover, SMN_{es} also increased with subsequent applications (Supplementary Information, Table S5). The net organic N mineralized in CON on Day 84 after Application 4 corresponded to 4.3 and 2.8% of the initial soil organic N content of the sandy loam and clay loam soil, respectively; the majority of the mineralization occurred during Application 1 (40 and 56% of the total mineralized N in the sandy loam and clay loam soil, respectively).

In the fertilized treatments, the recovery of applied ammonium as exchangeable NH_4-N in the soil at Day 0 averaged 86% in the sandy loam compared with recoveries of 64% (AS), 36% (HEI) and 42% (COW) in the clay loam soil (data not shown). Exchangeable NH_4-N concentrations decreased after each fertilizer application; it reached values that were similar to those of CON within one month at most. Ammonium-N decreased faster for HEI and COW than for AS, and was accompanied by a net increase in NO_3-N concentration during each application period (data not shown).

A different pattern in SMN_{es} was determined for AS in the sandy loam soil after Application 1. During Application 2, NH_4-N concentration decreased whereas NO_3-N remained stable; in subsequent applications, ammonium accumulated in the soil without concurrent increases in NO_3-N (data not shown). This is reflected by a clear decrease in SMN_{es} in Application 2 from 208 mg N kg^{-1} at Day 0 to about 130 mg N kg^{-1} at Days 41 and 84 (Figure 3c).

In all other treatments, SMN_{es} concentrations increased significantly (Table 4) during the time between application events (Figure 3d–h), although the increases were not always sizeable for HEI and COW. Increases across applications were also statistically significant (Supplementary Information, Table S5). Significant differences in SMN_{es} were noted between the soil types after two applications; SMN_{es} was larger in the sandy loam than in the clay loam soil (Supplementary Information, Table S6), at both the start and finish of each application period.

At Day 84 of each application, addition of ammonium with fertilizers raised non-exchangeable NH_4-N concentration in clay loam soil by 19 mg N kg^{-1} on average

(Figure 4). This quantity was similar for treatments in spite of the differing amounts of NH_4-N applied with fertilizers; indicated by different slopes for different fertilizers in Figure 4. The slopes of the fitted lines (Figure 4) show that the rank order of increase in non-exchangeable NH_4-N per unit of applied NH_4-N was HEI > COW > AS.

Recovery of N at Day 84

At the end of each application period, the amount of fertilizer-N that was recovered as SMN_t depended significantly on the soil × fertilizer × application interaction (Table 5). Similarly, there was a significant effect of the fertilizer × application interaction on the fraction of applied N recovered as non-exchangeable NH_4-N in the clay loam soil (Table 6). Table 7 gives the fitted trend in the recoveries of SMN_t across the four applications.

Nitrogen recovered as SMN_t in AS from the sandy loam soil (Figure 5a) decreased significantly between the first and last application (Table 7), and as incubation proceeded increasingly more applied N remained in an exchangeable NH_4-N form. In contrast, for the clay loam soil SMN_t recovery from the AS treatment ranged between 85 and 99%, and significantly more N accumulated in the soil as applications proceeded (Table 7), mostly as NO_3-N (Figure 5b). In the clay loam soil, an average of 20% of total applied N was also recovered as non-exchangeable NH_4-N (Figure 5b), which remained constant across applications (Supplementary Information, Table S7).

For the HEI treatment, exchangeable NH_4-N represented a small proportion (<1% of applied N) of SMN_t only in both soil types (Figure 5c,d), whereas NO_3-N averaged 27 and 9% of applied N in the sandy loam and clay loam soil, respectively. Recovery of HEI-applied N as SMN_t increased significantly across number of applications on the clay loam soil only (Table 7). Furthermore, in this treatment about 20% of applied N was recovered as non-exchangeable NH_4-N in the clay loam soil (Supplementary Information, Table S7).

For the COW treatment most of the applied N recovered as SMN_{es} at Day 84 (Figure 5e,f) was NO_3-N (35% and 15% of applied N in sandy loam and clay loam soil, respectively) and a small fraction only (<2% of applied N) was recovered as exchangeable NH_4-N. As for HEI, SMN_t for the COW treatment increased significantly with number of applications in the clay loam soil only (Table 7). Similar to AS and HEI, an average of 21% of applied N was measured as non-exchangeable NH_4-N in the clay loam soil (Table S7).

Nitrogen residual effects (NRE)

In the sandy loam soil, repeated applications of AS resulted in negative average NREs (Table 8); far more negative NREs were measured after Application 2 because of recovery of SMN_t. Conversely, in the clay loam soil the NRE of AS increased with number of applications. The HEI applied on sandy loam soil resulted in no clear patterns in NRE. The NRE values averaged zero even after three applications, whereas HEI applied to the clay loam soil produced small and stable NREs (2–5% of applied N) from Application 2 onwards (NRE_1). Estimated NREs for the COW treatment in the sandy loam soil fluctuated with no clear pattern as for HEI. The trend in NRE for COW in the clay loam soil was clearer; an average of 4 to 11% more slurry-N was recovered as SMN_t after two, three and four slurry applications.

Discussion

Soil mineral N dynamics

The two soils used in this experiment showed similar SMN_{es} dynamics. For all treatments, except AS on the sandy loam soil (see below), SMN_{es} concentration increased significantly during each 84-day application period (Figure 3, Table 4) because mineralization of soil organic N and nitrification of NH_4-N added to soil prevailed over microbial N immobilization.

The dynamics of soil NH_4-N and NO_3-N in the treatments considered (data not shown) were similar to those observed in soil to which AS and slurries were applied that quickly (within a few weeks) deplete NH_4-N because of nitrification and possibly immobilization by the microbial biomass (Calderón *et al.*, 2005; Bechini & Marino, 2009). Nitrification of ammonium applied with fertilizers and that derived from the mineralization of organic matter induced a net decrease in soil pH over time (Sørensen & Jensen, 1995; Sørensen, 1998). The decline was larger and faster in AS than in slurry-amended treatments (Figure 2c–h; Table S2), which might arise from the larger ammonium concentration in AS (Table 2), or the buffering capacity of slurries, or both (Sommer & Husted, 1995). Variation in pH was also significantly larger in the sandy loam than in the clay loam soil (Supplementary Information, Table S4) possibly because of the lower buffering capacity of the sandy loam soil. As incubation proceeded (and soil pH fell) for AS treatments, the rate of nitrification decreased. Aciego Pietri & Brookes (2008) studied soil with different pH amended with arginine, and also showed that the activity of nitrifiers was slow at pH less than about 6.1.

Repeated fertilizer additions combined with SMN_{es} that originated from mineralization of soil organic matter caused significantly more accumulation of SMN_{es} in both soil types (Supplementary Information, Table S5); however, the accumulation was significantly larger in the sandy loam than clay loam soil, especially after Application 2 (Supplementary Information, Table S6). This difference between the soil types arose not only from different rates of mineralization of native soil organic N (CON treatments in Figure 3a,b), but also for the AS, HEI and COW treatments. This was mainly because of differences in the availability of applied NH_4-N. Some of the fertilizer NH_4-N was not recovered in exchangeable form from the clay loam soil within two hours after application (Day 0, data not shown). This probably resulted from sequestration (fixation) of a consistent fraction of the ammonium added (36–64%, estimated by unrecovered NH_4-N) by clay minerals immediately after fertilizer application. Cavalli *et al.* (2015) showed previously that the same clay loam soil as the one used here could fix consistent amounts of NH_4-N (60 and 55% of N applied, when applied at rates of 70 and 140 mg NH_4-N kg^{-1}, respectively) within two days of AS application. This experiment also showed that the amount of fixed NH_4-N was directly proportional to that applied with both AS (Nõmmik & Vahtras, 1982; Kowalenko & Yu, 1996; Cavalli *et al.*, 2015) and slurries (Sowden, 1976).

During incubation, not all ammonium that was fixed initially was released. At the end of each application period (Day 84), an average of 19 mg N kg^{-1} was retained by clay minerals, independent of the amount of NH_4-N applied and fertilizer type (Figure 4). Therefore, the percentage of applied NH_4-N retained at Day 84 in non-exchangeable form was inversely proportional to the amount applied (HEI > COW > AS). This indicates that non-exchangeable NH_4-N release differed among the treatments (AS > COW > HEI), which was shown by the change in amounts of NH_4-N fixed that differed by treatment type at Day 0 to become similar at Day 84. It is plausible that a fraction of recently-fixed NH_4-N at Day 0 became strongly fixed and was not released during the ensuing 84 days (Nõmmik & Vahtras, 1982).

We expected the release of non-exchangeable NH_4-N to be larger than that observed, especially for HEI and COW, given that depletion of NH_4-N by microbial N immobilization and ammonium nitrification promotes ammonium defixation (Breitenbeck & Paramasivam, 1995). It is possible, however, that release of ammonium was partly inhibited by a large concentration of K^+ ions in the soil that were present either at the beginning of incubation (Table 1) or were applied with the manures (Table 2). Furthermore, it is likely that illite (Table 1) rather than smectites was responsible for ammonium fixation (Nõmmik & Vahtras, 1982). Fixation of ammonium (and potassium) probably occurred at the frayed edges of illite, which suggests collapse of the crystal lattice (Sawhney, 1972). Therefore, it is possible that ammonium retained in internal fixation sites was more inaccessible to microorganisms because the mineral interlayer could no longer expand (i.e. it remained collapsed) under our experimental conditions.

The SMN_{es} pattern of the AS treatment in the sandy loam soil after Application 1 (Figure 3c) was different from those of the other treatments. We suggest that after Application 2, low soil pH levels (which dropped below 4.6) inhibited the oxidation of NO_2^- to NO_3^- (second step of nitrification). If true, then the NO_2^- formed during the first step of nitrification might have been lost from the soil (Nelson, 1982; Pilegaard, 2013) after the formation of HNO_2. We know that chemodenitrification is enhanced by the instability of NO_2^- in acidic soil (Gerretsen & De Hoop, 1957; Islam *et al.*, 2008). By extension, the entire nitrification pathway might have been inhibited at Applications 3 and 4 because the concentration of NH_4-N in soil remained constant after both AS additions. Therefore, we do not discuss this treatment any further.

Recovery of N at Day 84 and estimated effects of residual N

The concentration of SMN_t in the clay loam soil amended with AS (Figure 5b) significantly increased over time (Table 7), which gave rise to NREs (Table 8) possibly because of the remineralization of microbially-immobilized N (Sørensen, 2004) or less N microbial immobilization because of progressively reduced C respiration as incubation proceeded (Cavalli *et al.*, 2014). On the contrary, the non-exchangeable NH_4-N fraction did not contribute to NRE (Supplementary Information, Table S7).

In slurry treatments, the SMN_t concentrations measured in both soil types at Day 84 corresponded roughly to the NH_4-N applied with HEI, and they were even smaller when applied with COW (dashed lines in Figure 5c–f). These results indicate that negligible net organic N was mineralized during HEI decomposition (4 and 6% of added N on the sandy loam and clay loam soil, respectively) and that considerable net N-immobilization occurred with COW (16% of applied N on both soil types). About 20% of applied slurries NH_4-N was in non-exchangeable form in the clay loam soil (Figure 5d,f). Therefore, less slurry N (–11 to –23%, and –14% to –29%, for HEI and COW treatments, respectively) was recovered as SMN_{es} in clay loam than sandy loam soil (Figure 5c–f).

The negligible and negative net N-mineralization measured in this experiment have also been obtained in other incubation studies of similar duration and on soil amended with relatively small C to organic N ratios (6–20 range) cattle slurries (Sørensen *et al.*, 2003; Bechini & Marino, 2009) and solid cattle manures (Thomsen & Olesen, 2000; Calderón *et al.*, 2005). The prevalence of microbial N immobilization over mineralization depends strongly on the composition of the slurry and on the mineralization of different components in the manure (Van Kessel *et al.*, 2000; Morvan & Nicolardot, 2009); the smaller is the content of N-poor fractions (e.g. cellulose and volatile fatty acids, VFA) the larger is the net N released during manure decomposition. Variation in immobilization of net slurry-N between HEI and COW treatments can be attributed to differences in their chemical characteristics (Table 2); the presence of VFA and the larger C to organic N ratio in the COW than HEI slurry might have promoted its greater microbial N immobilization (Kirchmann & Lundvall, 1993; Morvan *et al.*, 2006).

Repeated slurry applications led to significant increases in SMN_t (Figure 5c,f, Table 7) in the clay loam soil only, whereas the recovery of applied N as SMN_t in sandy loam soil fluctuated without a clear trend over time. Nevertheless, average NREs in the clay loam soil were modest, especially for the HEI treatment; they ranged between 2–5 and 4–11% of applied slurry-N for HEI and COW, respectively (Table 8). The absence of a steady rise in SMN_t with subsequent applications might have occurred because mineralization of the recalcitrant components takes a long time to be detected (Morvan *et al.*, 2006; Webb *et al.*, 2013). Our results for the sandy loam soil did not support the hypothesis that NREs increase with additional applications of slurry, with the accumulation of organic N and its continuous mineralization during the time after each application (Webb *et al.*, 2013). We consider that the discrepancy relates to mineralization that is too slow to produce an appreciable increase in SMN_t that can be separated from experimental variability. Nevertheless, it is noteworthy that AS in the clay loam soil produced NRE values similar to those of slurries. This suggests that under controlled conditions, remineralization of immobilized slurry NH_4-N primarily controlled NRE values rather than mineralization of recalcitrant organic-N components applied with the slurry. This is in accord with third-year residual effects, which have been observed in field experiments, that were similar for manures and mineral fertilizers (Schröder *et al.*, 2013; Webb *et al.*, 2013).

Conclusions

Results from this incubation study with four repeated slurry applications emphasize the importance of sequestration in the non-exchangeable form of ammonium applied to soil that contains ammonium-fixing clay minerals. In the clay loam soil, a fraction only of the ammonium fixed two hours after each slurry application was released during the ensuing 84 days. This caused a progressive accumulation (about 20 mg N kg^{-1} during each 84-day application period) of non-exchangeable ammonium that was independent of the amount applied. Because of the lack of clay fixation, significantly more soluble and exchangeable mineral N accumulated in the sandy loam than clay loam soil as the incubation proceeded.

This incubation study also confirmed the small net availability of slurry organic-N often observed in other experiments. In both soil types, final recoveries of slurry-N 84 days after four slurry applications were small for both slurries. Finally, net soil mineral N concentration was similar to or even less than the ammonium-N applied with slurries. Slurry-N mineralization averaged 5% for heifer slurry (HEI), whereas it was negative (–16%) for dairy cow slurry (COW).

The recovery of fertilizer N applied with both slurries increased significantly with subsequent applications for the clay loam only.

Our novel experimental set-up enabled us to eliminate many factors that interfere with the study of mineral nitrogen dynamics in soil and to measure the residual effect on clay loam soil. In the more coarse textured soil, however the effect appeared to proceed slowly and might take more time to become apparent.

Supplementary Information

In supplementary information, we provide the following tables:

Table S1. Summary of the analysis of variance for soil pH.

Table S2. Planned orthogonal polynomial contrasts of the linear effect of Sampling date on soil pH within Fertilizer × Application combinations.

Table S3. Planned orthogonal polynomial contrasts of the linear effect of the number of Applications on soil pH within different Soil × Fertilizer combinations.

Table S4. Planned orthogonal contrasts of Soil-type effect (sandy loam and clay loam) on soil pH within Application × Sampling date combinations.

Table S5. Planned orthogonal polynomial contrasts of the linear effect of the number of Applications on soluble plus exchangeable soil mineral nitrogen (SMN_{es}, mg N kg^{-1}) within different Soil × Fertilizer combinations.

Table S6. Planned orthogonal contrasts of Soil-type effect (sandy loam and clay loam) on soluble plus exchangeable soil mineral nitrogen (SMN_{es}, mg N kg^{-1}) within Application × Sampling date combinations.

Table S7. Planned orthogonal polynomial contrasts of the linear effect of Application on net non-exchangeable NH_4-N at Day 84 (% applied N) within Fertilizer.

Acknowledgements

We thank Professors Dr. Diedrich Steffens and Sven Schubert, and Mr. Roland Pfanschilling of the Justus Liebig Universität Gießen (Giessen, Hesse, Germany). They provided training and assistance on the methodology to determine non-exchangeable ammonium to Daniele Cavalli during his Ph.D.

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Tables

Table 1. Characteristics of the two soil types used in the incubation experiment.

Variable	Sandy loam soil	Clay loam soil
Sand/g kg^{-1}	666	448
Silt/g kg^{-1}	294	247
Clay/g kg^{-1}	40	305
Total C/g kg^{-1}	13.3	11.6
Total N/g kg^{-1}	1.45	1.39
Maximum NH_4^+ fixation capacity/ $cmol^+ kg^{-1}$	n.d.[a]	2.31
Cation exchange capacity/ $cmol^+ kg^{-1}$	6.5	25.2
Exchangeable Ca/mg kg^{-1}	340	2954
Exchangeable K/mg kg^{-1}	53	110
Exchangeable Mg/mg kg^{-1}	56	356
pH_W	6.70	6.80
Water content at –50 kPa (WC_{-50kPa})/ g H_2O kg^{-1}	110	205
Bulk soil		
Quartz/g kg^{-1}	n.d.	210
K-Feldspar/g kg^{-1}	n.d.	60
Plagioclase/g kg^{-1}	n.d.	120
Chlorite/g kg^{-1}	n.d.	180
Mica/Illite/g kg^{-1}	n.d.	150
Smectite/g kg^{-1}	n.d.	160
Kaolinite/g kg^{-1}	n.d.	120
Fraction < 2 µm		
Quartz/g kg^{-1}	n.d.	120
K-Feldspar/g kg^{-1}	n.d.	30
Plagioclase/g kg^{-1}	n.d.	40
Chlorite/g kg^{-1}	n.d.	190
Mica/Illite/g kg^{-1}	n.d.	160
Smectite/g kg^{-1}	n.d.	360
Kaolinite/g kg^{-1}	n.d.	100

[a]Not determined.

Table 2. Characteristics of the two slurries used in the incubation experiment.

Variable	Heifer slurry (HEI)	Dairy cow slurry (COW)
Dry matter/g kg^{-1}	39.0	81.7
Ash/g kg^{-1}	10.4	12.6
pH_W	8.82	7.97
K/g K kg^{-1}	2.5	1.9
Total C (TC) /g C kg^{-1}	13.9	34.9
Water soluble C/g C kg^{-1}	2.2	10.5
Volatile fatty acids/g C kg^{-1}	0.0	4.2
Total nitrogen (TKN)/g N kg^{-1}	1.36	3.98
Soluble N/g N kg^{-1}	0.51	2.49
Ammonium N (NH_4-N) /g N kg^{-1}	0.33	2.08
NH_4-N /TKN/g $100g^{-1}$	24.3	52.3
TC/TKN/g g^{-1}	10.2	8.8
TC/Organic N/g g^{-1}	13.5	18.4

Table 3. Summary of the analysis of variance for soluble plus exchangeable soil mineral nitrogen (SMN_{es}, mg N kg^{-1}).

Model	Degrees of Freedom	Mean square	*F*	*P*
Soil	1	74 053	454.0	<0.001
Fertilizer	3	508 154	3115.3	<0.001
Application	3	488 128	2992.6	<0.001
Sampling date	5	6534	40.1	<0.001
Soil × Fertilizer	3	59 224	363.1	<0.001
Soil × Application	3	2549	15.6	<0.001
Soil × Sampling date	5	705	4.3	<0.001
Fertilizer × Application	9	30 814	188.9	<0.001
Fertilizer × Sampling date	15	446	2.7	<0.001
Application × Sampling date	15	551	3.4	<0.001
Soil × Fertilizer × Application	9	12 662	77.6	<0.001
Soil × Application × Sampling date	15	479	2.9	<0.001
Fertilizer × Application × Sampling date	45	354	2.2	<0.001
Error	407	163		

Table 4. Planned orthogonal polynomial contrasts of linear effect of Sampling date on soluble plus exchangeable soil mineral nitrogen (SMN_{es}, mg N kg^{-1}) within Fertilizer × Application combinations. The fitted trend in SMN_{es} is given for each contrast within each 84-day period (± standard error).

Fertilizer[a]	Number of applications	Trend in SMN_{es} /mg N kg^{-1}[b]	*P*
CON	1	22 ± 5	<0.001
	2	16 ± 5	0.003
	3	14 ± 5	0.012
	4	12 ± 6	0.040
AS	1	18 ± 5	0.001
	2	–21 ± 5	<0.001
	3	20 ± 6	<0.001
	4	18 ± 5	0.001
HEI	1	28 ± 5	<0.001
	2	26 ± 5	<0.001
	3	23 ± 6	<0.001
	4	15 ± 6	0.007
COW	1	21 ± 5	<0.001
	2	20 ± 5	<0.001
	3	19 ± 6	0.001
	4	26 ± 5	<0.001

[a]CON, control with water; AS, control with ammonium sulphate; HEI, heifer slurry; COW, dairy cow slurry.
[b]Estimated increase or decrease in SMN_{es} from Day 0 to Day 84.

Table 5. Summary of the analysis of variance for net total soil mineral nitrogen at Day 84 (SMN_t, % applied N).

Model	Degrees of freedom	Mean square	*F*	*P*
Soil	1	3967	774.7	<0.001
Fertilizer	2	9381	1832.0	<0.001
Application	3	66	13.0	<0.001
Soil × Fertilizer	2	3726	727.6	<0.001
Soil × Application	3	213	41.7	<0.001
Fertilizer × Application	6	251	48.9	<0.001
Soil × Fertilizer × Application	6	118	23.1	<0.001
Error	42	5		

Table 6. Summary of the analysis of variance for net non-exchangeable NH_4-N at Day 84 (% applied N).

Model	Degrees of freedom	Mean square	*F*	*P*
Fertilizer	2	0.1	0.11	0.897
Application	3	60.7	45.66	<0.001
Fertilizer × Application	6	6.0	4.52	0.004
Error	22	1.3		

Table 7. Planned orthogonal polynomial contrasts of the linear effect of Application on net total soil mineral nitrogen at Day 84 (SMN_t, % applied N) within Soil × Fertilizer combinations. The fitted trend of SMN_t from Application 1 to Application 4 is given for each contrast (± standard error).

Fertilizer [a]	Soil	Trend in net SMN_t /% of applied N[b]	*P*
AS	Sandy loam	–20.2 ± 2.0	<0.001
	Clay loam	12.2 ± 2.2	<0.001
HEI	Sandy loam	–0.3 ± 2.2	0.907
	Clay loam	4.7 ± 2.0	0.020
COW	Sandy loam	1.7 ± 2.2	0.435
	Clay loam	15.6 ± 2.2	<0.001

[a]AS, control with ammonium sulphate; HEI, heifer slurry; COW, dairy cow slurry.
[b]Estimated increase or decrease in SMN_t at Day 84 from Application 1 to Application 4.

Table 8. Nitrogen residual effects (NRE, % N applied) at Day 84 after one, two and three additions of ammonium sulphate, heifer slurry and dairy cow slurry to the sandy loam and clay loam soil. Mean ± standard error.

NRE	Estimate	Soil: Sandy loam, Fertilizer [a]: AS	Sandy loam: HEI	Sandy loam: COW	Clay loam: AS	Clay loam: HEI	Clay loam: COW
NRE1	Addition 2 – Addition 1	–37.9 ± 1.6	4.9 ± 0.6	8.3 ± 3.9	–4.7 ± 3.7	4.1 ± 2.2	6.0 ± 2.7
	Addition 3 – Addition 2	9.3 ± 1.9	0.3 ± 0.6	–6.7 ± 2.2	8.6 ± 4.2	–1.3 ± 1.3	7.1 ± 0.7
	Addition 4 – Addition 3	5.4 ± 2.1	–5.5 ± 0.4	5.2 ± 1.1	6.1 ± 2.3	2.2 ± 0.9	–2.3 ± 0.9
	Average	–7.8 ± 1.9	–0.1 ± 0.5	2.3 ± 2.6	3.3 ± 3.5	1.7 ± 1.6	3.6 ± 1.7
NRE2	Addition 3 – Addition 1	–28.7 ± 1.8	5.1 ± 0.5	1.6 ± 3.5	3.9 ± 2.2	2.8 ± 2.1	13.1 ± 2.7
	Addition 4 – Addition 2	14.6 ± 2.0	–5.2 ± 0.5	–1.5 ± 1.9	14.7 ± 3.8	0.9 ± 1.0	4.8 ± 0.8
	Average	–7.0 ± 1.9	0.0 ± 0.5	0.1 ± 2.8	9.3 ± 3.1	1.9 ± 1.7	9.0 ± 2.0
NRE3	Addition 4 – Addition 1	–23.3 ± 1.9	–0.3 ± 0.4	6.8 ± 3.4	10.0 ± 1.2	5.0 ± 2.0	10.8 ± 2.7

[a]AS, control with ammonium sulphate; HEI, heifer slurry; COW, dairy cow slurry.

Figure captions

Figure 1. The experiment evaluated the application of fertilizers (water, ammonium sulphate, heifer and dairy cow slurries) to two soil types (sandy loam and clay loam) for one, two, three or four times. After each application, measurements were made over 84 days. Each arrow represents a fertilizer application.

Figure 2. Soil pH following repeated applications of water, ammonium sulphate, heifer slurry and dairy cow slurry to the sandy loam and clay loam soil. S.E., standard error.

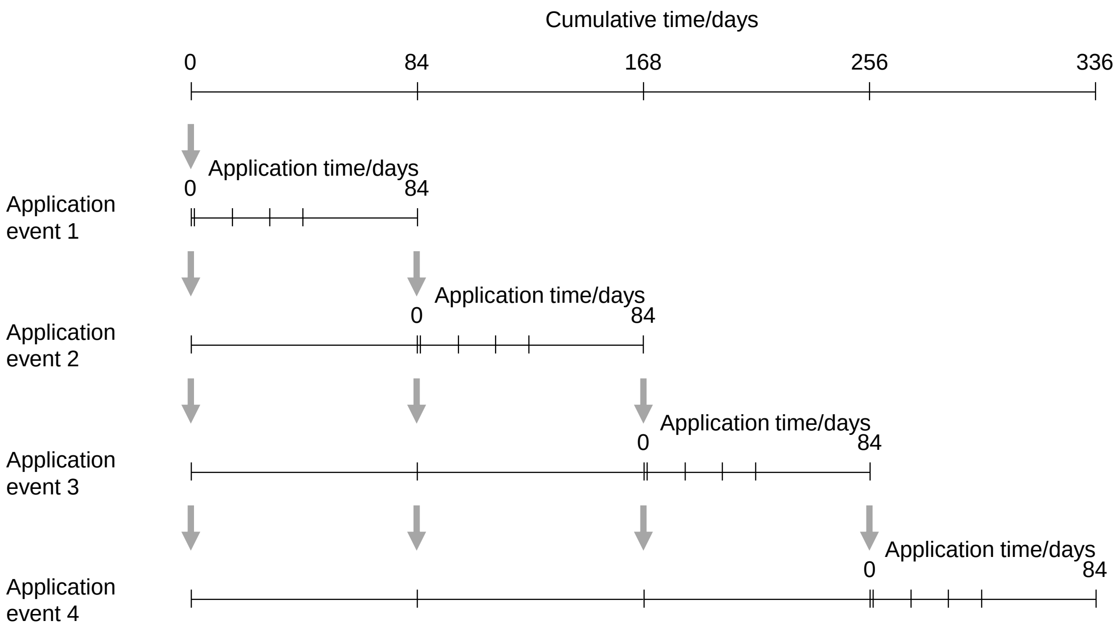
Figure 3. Soluble plus exchangeable soil mineral nitrogen concentration (SMN_{es}, mg N kg^{-1}) following repeated applications of water, ammonium sulphate, heifer slurry and dairy cow slurry to the sandy loam and clay loam soil. S.E., standard error.

Figure 4. Net non-exchangeable NH_4-N concentration (mg N kg^{-1}) measured at Day 84 following each of four applications (A1–4) of ammonium sulphate (AS), heifer slurry (HEI) and dairy cow slurry (COW) to the clay loam soil. S.E., standard error.

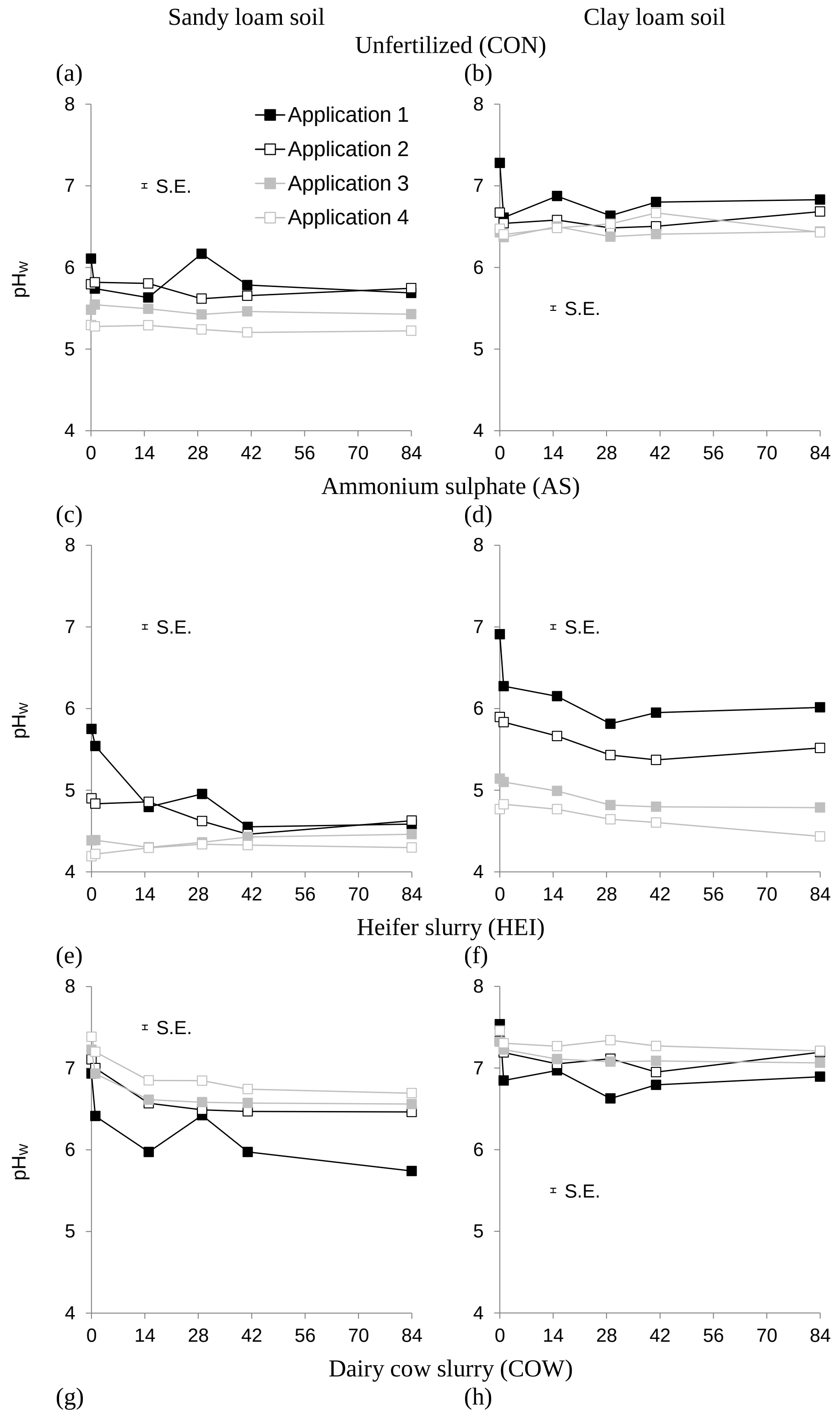
Figure 5. Nitrogen recovery (as SMN_t = exchangeable NH_4-N + NO_3-N + non-exchangeable NH_4-N) at Day 84 following repeated applications (A1–4) of

ammonium sulphate (AS), heifer slurry (HEI) and dairy cow slurry (COW) to the sandy loam and clay loam soil. The horizontal dashed line represents the NH_4-N to total N ratio in slurries. S.E., standard error of SMN_t.

Figure 1


Cumulative time/days
0
84
168
256
336
Application time/days
0
84
Application event 1
Application time/days
0
84
Application event 2
Application time/days
0
84
Application event 3
Application time/days
0
84
Application event 4

679 **Figure 2**


Sandy loam soil
Clay loam soil
Unfertilized (CON)
(a)
(b)
Application 1
Application 2
Application 3
Application 4
S.E.
pHw
Ammonium sulphate (AS)
(c)
(d)
Heifer slurry (HEI)
(e)
(f)
Dairy cow slurry (COW)
(g)
(h)
0
14
28
42
56
70
84
4
5
6
7
8

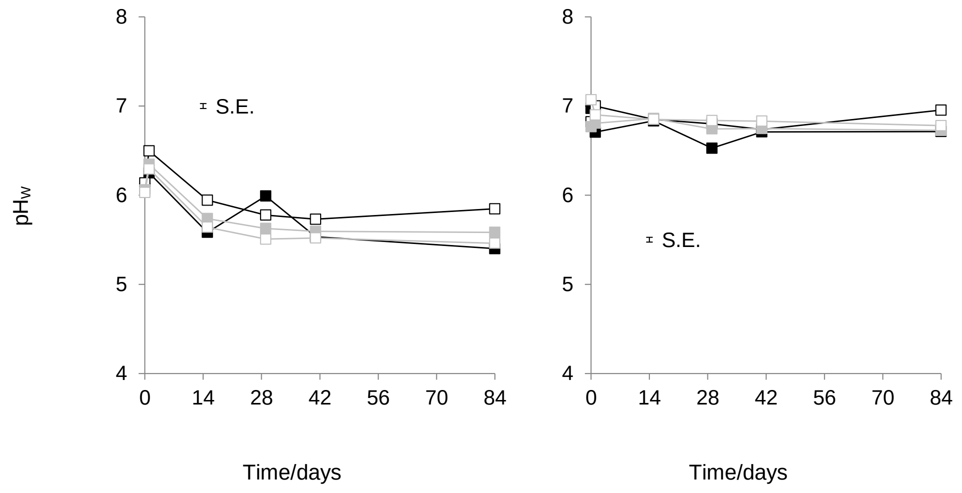
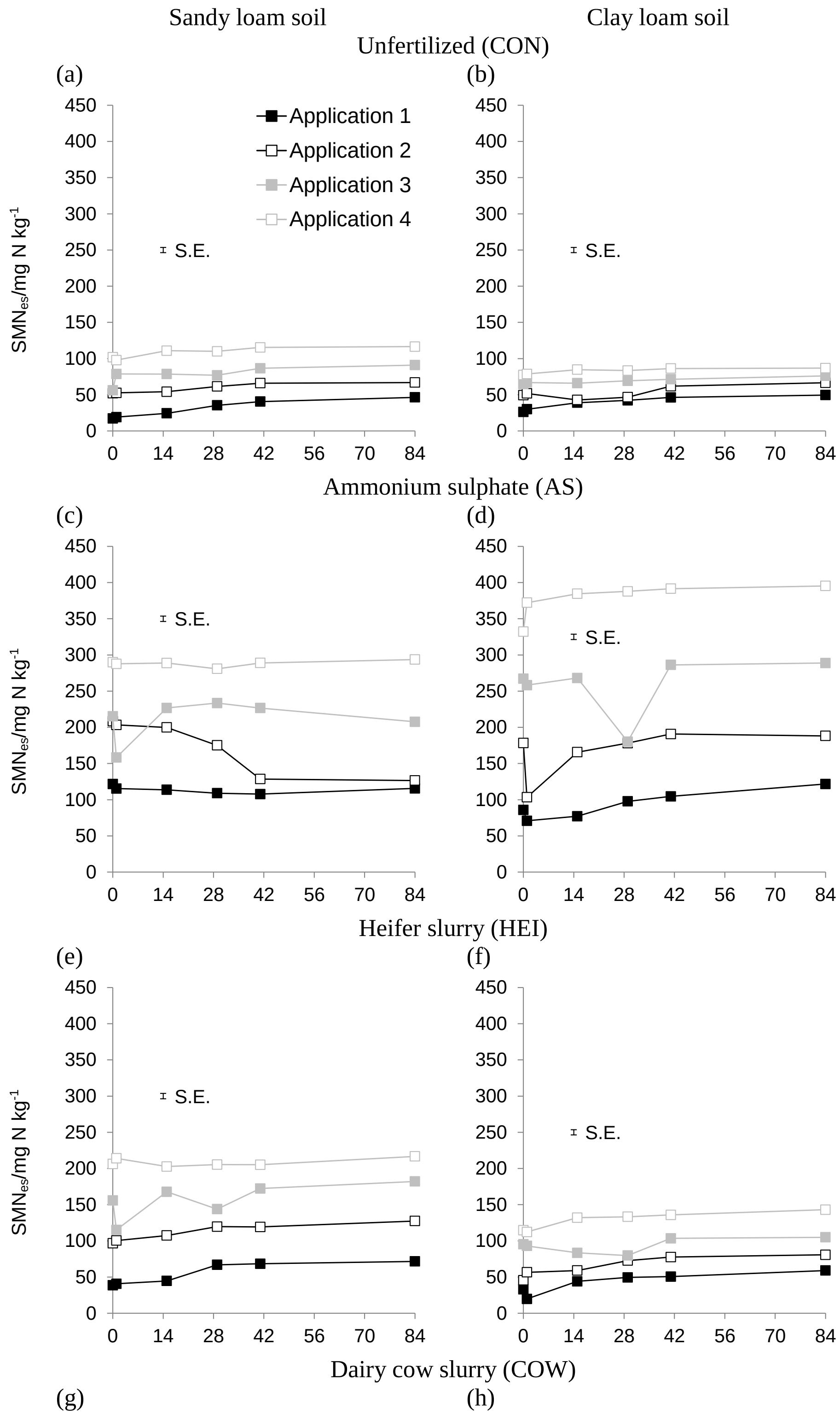

pHW
8
7
6
5
4
S.E.
0
14
28
42
56
70
84
Time/days
8
7
6
5
4
S.E.
0
14
28
42
56
70
84
Time/days

Figure 3


Sandy loam soil
Clay loam soil
Unfertilized (CON)
(a)
(b)
Application 1
Application 2
Application 3
Application 4
S.E.
SMNes/mg N kg-1
0
14
28
42
56
70
84
0
50
100
150
200
250
300
350
400
450
Ammonium sulphate (AS)
(c)
(d)
Heifer slurry (HEI)
(e)
(f)
Dairy cow slurry (COW)
(g)
(h)

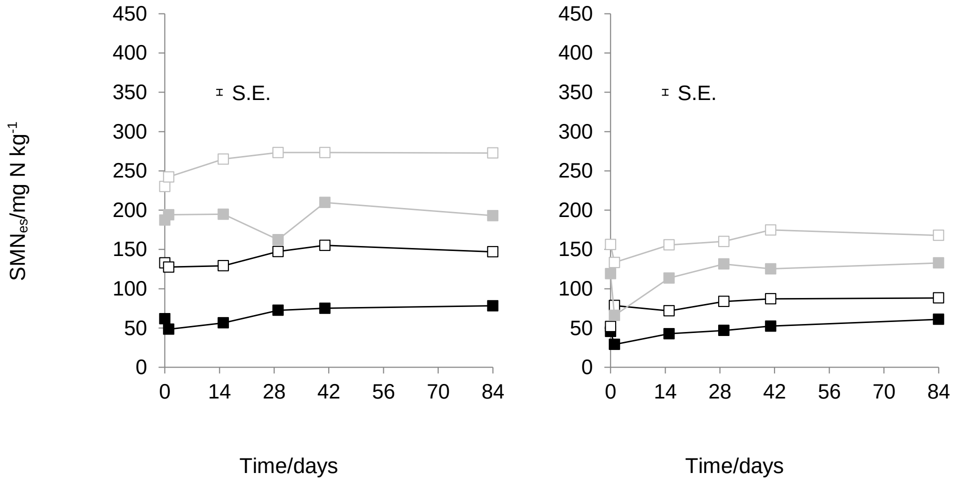
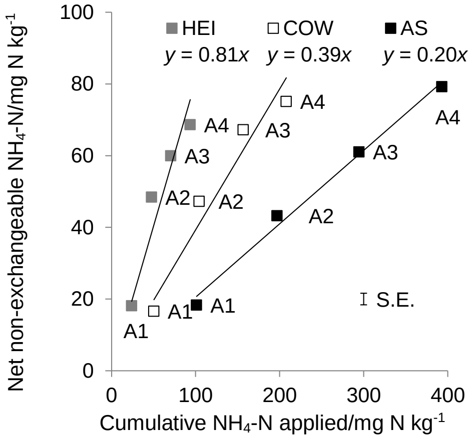
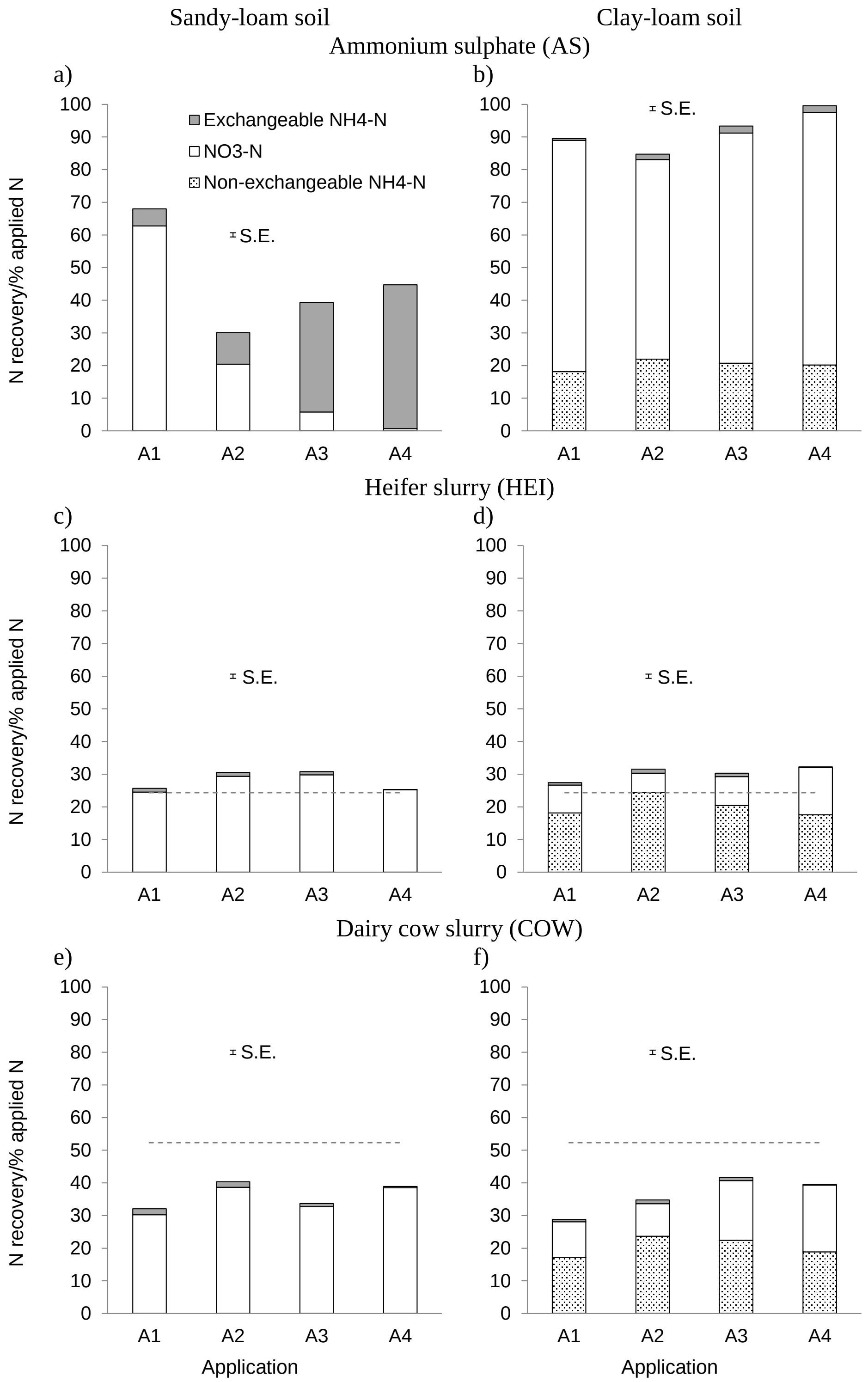

SMNes/mg N kg-1
S.E.
S.E.
Time/days
Time/days

Figure 4



HEI
COW
AS
y = 0.81x
y = 0.39x
y = 0.20x
A1
A2
A3
A4
S.E.
Net non-exchangeable NH4-N/mg N kg-1
Cumulative NH4-N applied/mg N kg-1
0
20
40
60
80
100
200
300
400

Figure 5


Sandy-loam soil
Clay-loam soil
Ammonium sulphate (AS)
a)
b)
Exchangeable NH4-N
NO3-N
Non-exchangeable NH4-N
S.E.
N recovery/% applied N
A1
A2
A3
A4
Heifer slurry (HEI)
c)
d)
Dairy cow slurry (COW)
e)
f)
Application