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# Process rates of nitrogen cycle after harvesting in no-till and ploughed agricultural clay soils

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## Keywords

zero till, conventional tillage, nutrient, gross rate, stable isotope, agriculture

## Abstract

No-till is considered an advantageous agricultural practice for the environment because soil erosion is decreased compared to ploughed soil. However, for an overall evaluation of the benefits and disadvantages of this crop production method, it is also important to understand the soil nutrient cycling in no-till fields. Little is known about how the gross rates of the different processes of nitrogen (N) cycle are affected by tillage practices in agroecosystems in a northern cold climate. Our study was designed to obtain information about gross soil N process rates in boreal no-till and moldboard ploughed spring barley fields after autumn harvesting. Gross organic N mineralization into ammonium ( $\text{NH}_4^+$ ) and  $\text{NH}_4^+$  immobilization were the most important N transformation processes in the soil and their rates were higher in no-till soil than in ploughed soil. Regardless of the higher mineralization rate, the gross rate of  $\text{NH}_4^+$  oxidation into nitrate ( $\text{NO}_3^-$ ) was clearly lower in no-till soil. This was explained by higher  $\text{NH}_4^+$  immobilization in no-till soil. The lower  $\text{NH}_4^+$  oxidation rate in no-till soil decreased the risk for  $\text{NO}_3^-$  leaching, which supports the promulgated environmental benefits of this practice.

## Introduction

Understanding the effects of different agricultural soil management practices on factors that eventually affect soil quality and the quality and yield of crops is of major importance environmentally and economically. Nitrogen (N) is the most important limiting nutrient for crop production (Fageria and Baligar 2005). Apart from being an important nutrient for plants and other organisms however, N has also negative environmental consequences. The N load to aquatic systems has increased in recent decades (Galloway *et al.* 2003), and the reduction of nitrous oxide (N<sub>2</sub>O) emissions from agriculture is an important goal for climate change mitigation (Paustian *et al.* 2016). Thus, it is important to get a better understanding of soil N cycle transformation processes and the factors that drive them under different soil management regimes. The ensemble of the N cycle in soils is complex, since there are many processes and also many kinds of organisms involved. A comprehensive understanding of the topic is needed, although knowledge of the N cycle processes and steps has improved over the last decades. For example, it is now known that in addition to bacteria, archaea are also involved in the oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) (Leininger *et al.* 2006; Nicol and Schleper 2006).

Results about N cycle processes in no-till soil ecosystems can be quite variable and depend on climatic factors, the success of the farming practice, on soil characteristics, and also the crop species (Soane *et al.* 2012). The soil management affects the physical characteristics of soils, which include for example soil moisture, soil temperature and the location of soil organic matter (SOM) in the soil profile. These factors in turn affect soil microbes and further N cycle processes. Soil management also has more direct environmental impacts. No-till is considered an environmentally better soil practice than conventional tillage because no-till practice prevents erosion by increasing protective crop residue coverage on soil surface and reduces sediment discharge (Matisoff *et al.* 2002; Merrill *et al.* 2006; Montgomery 2007). Possible disadvantages of no-till are that drilling may be delayed for spring sown crops in cool climates because of the excessively high moisture content and the relatively low temperature of the surface soil, and seeds being in close contact with crop plant residues in no-till soils increase the risk for fungal infection in wet soils (Soane *et al.* 2012). Crop yields in no-till soils in the northern areas are usually lower in comparison to ploughed soils especially in the first years after adopting this soil management approach (Soane *et al.* 2012).

It is well known that no-till increases surface soil carbon (C) content (Franzluebbers 2008; Dong *et al.* 2012; Gómez-Rey *et al.* 2012; Virto *et al.* 2012), since most of the organic residues are deposited and incorporated in the surface layer (Porporato *et al.* 2003). However, the total C pool of the entire soil profile might not increase, which is important to consider when evaluating different soil practices in terms of C sequestration and climate change mitigation measures. No-till or reduced till soils often have a higher C content in the surface soil layers but have correspondingly less C in their deeper soil layers (Luo *et al.* 2010; Abdollahi and Munkholm 2014; Neugschwandtner *et al.* 2014; Powlson *et al.* 2014; Huang *et al.* 2015; Singh *et al.* 2015; Valboa *et al.* 2015). Sheehy *et al.* (2015) found that soil organic carbon in boreal agroecosystems is redistributed in no-till to more stable particles in comparison to moldboard ploughed soils. Our experimental area was also included in the study published by Sheehy *et al.* (2015). A vertical distribution have been observed also in microbial biomass C, so more of it in the surface soil than in deeper layers (Oorts *et al.* 2007a; Abdollahi and Munkholm 2014).

Similar trend in total soil N or organic N distribution as in surface soil C distribution can occur in no-till / reduced till compared to moldboard ploughed soils, as soil N content in no-till soils is often higher in surface soil than in deeper layers (Oorts *et al.* 2007a; Abdollahi and Munkholm 2014; Neugschwandtner *et al.* 2014; Valboa *et al.* 2015). Neugschwandtner *et al.* (2014) found that specifically the mineralizable N is higher in no-till soils than in moldboard ploughed soils at 0–10 cm.

Dry bulk density of topsoil is usually higher in no-till soils than in ploughed soils in humid regions (Regina and Alakukku 2010; Sipilä *et al.* 2012). However, in the first few centimeters of the soil surface the bulk density can provide conflicting results (Oorts *et al.* 2007a). Surface soil moisture is usually increased in no-till in humid regions (Merrill *et al.* 2006; Oorts *et al.* 2007a; Rochette 2008; Almaraz *et al.* 2009; Regina and Alakukku 2010; Soane *et al.* 2012), and the water filled pore space on surface soil in the growing season is increased (Dharmakeerthi *et al.* 2004; Oorts *et al.* 2007b; Ball *et al.* 2008). Soil moisture controls the soil C and N cycles by affecting decomposition, leaching and plant uptake, and also the soil C/N ratio affects the organic matter decomposition rate (Porporato *et al.* 2003). The influence of soil moisture on the N cycle processes is mainly related to aeration because wet anoxic conditions prevent bacteria from conducting aerobic oxidation of

N compounds, although insufficient water content also reduces microbial activity (Porporato *et al.* 2003). In contrast to soil moisture, no-till method may decrease surface soil temperatures in humid regions in the growing season (Dharmakeerthi *et al.* 2004; Almaraz *et al.* 2009) and especially in the spring time (Soane *et al.* 2012) as surface mulch decreases insolation of the top soil (Triplett and Dick 2008). Soil temperature is, however, usually a less important factor in the N cycle than soil moisture, especially on a daily time scale (Porporato *et al.* 2003).

An increase in N<sub>2</sub>O emissions is an important potential disadvantage of no-till especially with regard to clayey soils in a humid climate (Six *et al.* 2004; Gregorich *et al.* 2005; Sheehy *et al.* 2013). The N<sub>2</sub>O emissions are connected to the interaction between soil structure and climate factors, which affect soil aeration (Gregorich *et al.* 2005). The risk of increasing N<sub>2</sub>O emissions is higher in the northern areas which tend to have high soil moisture content (Soane *et al.* 2012). Sheehy *et al.* (2013) found that although N<sub>2</sub>O emissions are increased in dense structured clayey no-till soils compared to conventional till soils due to increased soil moisture and poor aeration, the situation might be the opposite in coarse structured soils. This view is supported by Rochette (2008) who observed that N<sub>2</sub>O emissions are generally increased in poorly aerated, but do not increase from better aerated no-till soils. The situation of N<sub>2</sub>O emissions may alter in time for the benefit of no-till method, i.e. N<sub>2</sub>O emissions are reported to decrease after about a decade of adopting no-till practice (Six *et al.* 2004). The potential problem of increasing N<sub>2</sub>O emissions could also be mitigated by adapting the appropriate N fertilization practices, as indicated by Halvorson and Del Grosso (2012). Those authors reported that higher N<sub>2</sub>O fluxes were emitted from a no-till cornfield that had been fertilized with granular urea than when it had been fertilized with polymer-coated urea.

Studies of process-specific gross N transformation rates of the different simultaneous steps of the N cycle in different agroecosystems are limited. In humid climate, the differences in the gross N transformation rates in surface soils for no-till (Muruganandam *et al.* 2010; Hu *et al.* 2013), and in conservation till (8 years under no-till and 6 years under reduced till) soils (Gómez-Rey *et al.* 2012) have been observed. However, those studies were not been conducted on northern, cold climate agroecosystems. Our study was designed to fill this knowledge gap by conducting a <sup>15</sup>N tracing experiment in no-till and moldboard ploughed spring barley (*Hordeum vulgare* L.) field in Finland. We hypothesized that the gross N transformation rates are in general

higher in the no-till than in the ploughed surface soil. Our reasoning for this hypothesis was because the no-till surface soil has higher SOM content, which may cause higher activity of microbes involved in the soil N cycle processes.

## Materials and methods

### Experimental area

The study was performed within a field in the Natural Resources Institute Finland (Luke) in Jokioinen in Southern Finland (60°49'N, 23°28'E). The Luke study site (160 \* 60 m) was established in 2001. The soil type of the experimental site is Vertic Luvisol Stagnosol (Hypereutic, Clayic) (FAO 2014). Four no-till and four autumn moldboard ploughed field plots (each 25 \* 10 m) of the study site were used in the experiment. The study site had 16 plots in 2 lines (i.e. 32 plots in total), and it included also treatment plots that were not included in this study. The location of the study site treatment plots were randomized. The two field lines were separated by a 10 m wide zone, and the 16 plots were side by side. The plots included in this study were separated by 2–4 other plots because of the randomization. We studied *in situ* soil gross N transformation process rates in September 2010 after harvesting the spring barley (*Hordeum vulgare* var. *Annabell*) that was grown on the experimental site. Harvesting was performed on August 23<sup>rd</sup> 2010. Sampling was performed before the autumn moldboard ploughing of the till plots. There was no heavy rain during the experiment, but after incubation day 2 (see description for incubation under heading “Labeling and incubation”) there was occasionally light rain showers. The mean with standard deviations (SD) for surface soil water content (0–5 cm) was similar for both soil treatments, 27.1 volume% (SD 6.4) in no-till and 29.1 volume% (SD 5.7) in ploughing. The soil moisture slightly increased after incubation day 2 due to the light rain showers.

The cultivation methods used in the experimental field were as those of typical local practices (Mikkola *et al.* 2005). The no-till plots had been drilled in the spring by using a seed-drill (double disk coulters), which placed the fertilizer and seeds in the same row (Fig. 1) and to the same depth. The row space was 14.0 cm and the sowing depth about 3–4 cm. The other plots were moldboard ploughed to 20–25 cm depth in previous autumn and then sown in spring by combined rotary harrowing and drilling (one-pass method, combined drill).

Seeds (shoe coulters) and fertilizer were placed at the same time to separate rows. The row space of seed and fertilizer coulters was 12.5 and 25.0 cm, respectively. Sowing depth was about 4–5 cm and 2 cm deeper for fertilizer. Before drilling, the ploughed soil furrows were leveled by harrowing to 3–4 cm depth to make the soil more friable.  $\text{NH}_4\text{NO}_3$  fertilizer (NPK 27-0-1) was added  $370 \text{ kg ha}^{-1}$  for both types of soil treatment plots. The target seed rate was 500 viable seeds per  $\text{m}^2$  ( $210 \text{ kg ha}^{-1}$ ).  $\text{pH}_{\text{H}_2\text{O}}$  was measured by Sipilä *et al.* (2012) in 2009 in the same experimental area as ours (0–5 cm), where the pH values were 5.8 in no-till and 5.9 for ploughed soil.

### Labeling and incubation

We used a  $^{15}\text{N}$  pool dilution and tracing technique to quantify gross N transformation process rates in soils. In general, all the individual processes of the N cycle have an effect on all the other N cycle gross process rates directly or indirectly (Rütting *et al.* 2010). Therefore, a complex  $^{15}\text{N}$  tracing model and a mirror  $^{15}\text{N}$  labeling ( $^{15}\text{N}$  labeled ammonium nitrate;  $^{15}\text{NH}_4\text{NO}_3$  and  $\text{NH}_4^{15}\text{NO}_3$ ) was used to differentiate the specific process of the N cycle (Müller *et al.* 2007). The tracing model is based on altering  $^{15}\text{N}/^{14}\text{N}$  ratios that occur as a result of the ongoing processes of the N cycle. Thus, when  $\text{NH}_4^+$  is produced by natural processes in  $^{15}\text{NH}_4\text{NO}_3$  labeled soil, the excess  $^{15}\text{N}$  of the  $\text{NH}_4^+$  pool will be diluted, and along with nitrification the  $^{15}\text{N}/^{14}\text{N}$  ratio in  $\text{NO}_3^-$  increases. Similar reasoning applies when the nascent  $\text{NO}_3^-$  is produced in  $\text{NH}_4^{15}\text{NO}_3$  labeled soil, so the excess  $^{15}\text{N}$  of  $\text{NO}_3^-$  will be diluted. An assumption of the model is that there is no isotopic discrimination. Although this is not exactly true, discrimination does not affect the results due to the high  $^{15}\text{N}$  enrichment (Rütting 2012). The other advantage of using highly enriched  $^{15}\text{N}$  salt is that only a very small amount of the N is needed to achieve sufficient labeling. This was important in our experimental site as the inorganic N concentrations of the experimental soils were low.

The  $^{15}\text{N}$  label was added *in situ* using a Virtual Soil Core approach (Rütting *et al.* 2011; Staelens *et al.* 2012) for keeping the soil as undisturbed and conditions as natural as possible. On September 6<sup>th</sup> 2010, labeling points were marked out by pegging, and any loose organic material or weeds were removed from these points.  $^{15}\text{N}$  labeling was performed at 16 days after harvesting, on September 8<sup>th</sup>, when each labeling point received  $83 \mu\text{g N}$  in  $\text{NH}_4\text{NO}_3$  solution (7 ml per each point). Half of the points (40 points) received the solution with  $^{15}\text{N}$ - $\text{NH}_4^+$  enrichment ( $^{15}\text{NH}_4\text{NO}_3$ ), and the other half (40

points) received  $^{15}\text{N-NO}_3^-$  enriched ( $\text{NH}_4^{15}\text{NO}_3$ ) solution, both with a  $^{15}\text{N}$  fraction of 98%. All the soil sample points were injected within one day and they were left intact until soil sampling at 0, 1, 2, 5 and 9 days of incubation. The  $^{15}\text{NH}_4\text{NO}_3$  label points for the different incubation times were arranged in a line that intersected with the direction of the coulter tracks within each field plot (Fig. 1). The  $\text{NH}_4^{15}\text{NO}_3$  label points were arranged 1 m away from the line of the  $^{15}\text{NH}_4\text{NO}_3$  label points. The labeling and sampling points were always in the unfertilized lines between the sowing rows. There were 80 labeling points in total comprising: the four no-till plots and four ploughed plots, and two  $^{15}\text{N}$  labels and five incubation times for each plot.

Labeling solution was delivered by spinal needles at a depth of 0–5 cm so that 1 ml of solution was injected through a plate vertically and evenly and this was done 7 times for each sample point as 7 equal dots. Six of the dots were arranged in a circle ( $\text{Ø}$  4.0 cm) and the 7<sup>th</sup> dot was placed in the middle of that circle. The exact time of finishing the latest of the 7 injections was recorded for each sample point. The plate was pulled-up after labeling, but pegs that had been placed at the corners of the plate to mark the points, were left in the ground for later recognition. We loosely put back the straw and other crop residues that were removed beforehand from the labeling points to prevent the soil from drying out. All tools and equipment for  $^{15}\text{NH}_4\text{NO}_3$  and for  $\text{NH}_4^{15}\text{NO}_3$  label solutions and samples were kept strictly separate for the whole experiment to prevent cross-contamination of the labels.

### Sampling and laboratory analyses

After 0, 1, 2, 5 and 9 days of incubation, the labeled soil was sampled by an auger (0–5 cm depth). Before soil sampling, the straw remains were removed again from the soil surface. A plate with a hole in the middle was placed onto the ground within the space that had been previously marked out as labeling point by sticks. In this way the exact labeled points of the soil were sampled. The auger diameter was 6 cm and the auger was thoroughly cleaned between successive samplings to avoid cross-contamination from the previous sampling. On incubation day 0, the soil was sampled from the ground almost immediately after the injection. We waited for 10–15 minutes before sampling to let those samples stabilize. Each unbroken soil core was put into a plastic bag and taken to the laboratory for further processing, four samples at a time.



Each sample was sieved (4 x 4 mm mesh size) and homogenized immediately after being taken to the laboratory and 100 g of fresh soil was weighted into a centrifuge bottle. A 200 ml quantity of 2 M KCl solution was added into the contents of the bottle in order to stop the ongoing processes in the soil. The samples were shaken for 1 hour (140 rpm) and centrifuged at 1500 rpm (+20°C) for 20 minutes. The KCl blanks without soil were also shaken. Samples and KCl blanks were then filtered through Whatman Glass microfibre filters (GF/D Cat no. 1823-125). The rest of the sieved and homogenized soil sample was saved for bulk soil analyses. The time between sampling and KCl extraction was a maximum of one hour.

Later on, the extracted samples were prepared for the  $\text{NH}_4^+\text{-N}$  concentration analyses with a spectrophotometer (Anonymous 1976).  $\text{NO}_3^-\text{-N}$  was analyzed by hydrazine reduction with Lachat instruments (QuikChem 12-107-04-1-E).  $^{15}\text{N}$  enrichment in  $\text{NH}_4^+$  and in  $\text{NO}_3^-$  pools of the extracts was determined by methods based on the generation of  $\text{N}_2\text{O}$  gas phase for Isotope Ratio Mass Spectrometry (IRMS) (Stevens and Laughlin 1994; Laughlin *et al.* 1997). The sieved bulk soil samples were oven dried at 105 °C for 24 hours to determine soil water content. Dry bulk density was calculated by dividing the dry weight of a soil core by its volume and transferring the result into  $\text{kg m}^{-3}$ . Dried soil was ground in a ball mill (Retsch MM200; ball diameter 12 mm; 4 minutes; 1200 rpm). C-N analyses were performed with automated dry combustion (Leco CNS-2000) and bulk soil total  $^{15}\text{N}$  was determined by an EA-IRMS (Elemental Analyzer Isotope Ratio Mass Spectrometer).

## Data analyses

Before statistical analyses and calculating parameter means and standard deviations, the individual sample results differing only by the label ( $^{15}\text{NH}_4\text{NO}_3$  and  $\text{NH}_4^{15}\text{NO}_3$ ), were pooled mathematically by calculating the mean for both samples. Thus, there were 40 values per parameter after the calculations: two soil treatments, four plots and five incubation days. For modeling, non-pooled data was used.

Differences of soil properties between the no-till and ploughed treatments were tested by one-way repeated measures of analysis of variance (ANOVA). An interaction was found between treatments and incubation times for  $\text{NH}_4^+\text{-N}$  concentrations. Therefore, the differences of the  $\text{NH}_4^+\text{-N}$  concentrations between soil treatments were tested separately at every incubation time with

one-way ANOVA. The statistical tests were performed with IBM SPSS statistics 22 (Anonymous 2013).

The process-specific gross N transformation rates in no-till and ploughed soils were quantified with a numerical tracing model *Ntrace* based on Monte Carlo Sampling (Müller *et al.* 2007). The general mathematical notation of the model is specified by Müller *et al.* (2004). The final model setup used for data analysis contained seven N transformation processes, namely: mineralization of organic N to  $\text{NH}_4^+$  ( $M_{\text{Norg}}$ ), release of adsorbed  $\text{NH}_4^+$  ( $R_{\text{NH}_4}$ ), immobilization of  $\text{NH}_4^+$  to organic N ( $I_{\text{NH}_4}$ ), loss flux of  $\text{NH}_4^+$  ( $L_{\text{NH}_4}$ ), oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  ( $O_{\text{NH}_4}$ ), release of stored  $\text{NO}_3^-$  ( $R_{\text{NO}_3}$ ) and loss flux of  $\text{NO}_3^-$  including leaching, gaseous N loss and lateral diffusion ( $L_{\text{NO}_3}$ ). The two loss fluxes were included as the labeling was conducted in an open system and losses of the  $^{15}\text{N}$ , e.g. by lateral diffusion, needed to be taken into consideration. Data analysis was conducted separately for no-till and for ploughed soil data and included the data of the exact incubation times,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations,  $^{15}\text{N}$  abundance of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and bulk  $^{15}\text{N}$  excess. The principle of the modeling process is to change the initial parameter values to achieve a close fit between modeled and measured values of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations and  $^{15}\text{N}$  excess of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . As a result, optimized model parameters are obtained, which provide the gross N transformation rates. The modeled results are presented as  $\mu\text{g N (g dry soil)}^{-1} \text{d}^{-1}$ ) and also as a result of a per area calculation ( $\text{mg N m}^{-2} \text{d}^{-1}$ ).

## Results

The  $\text{NH}_4^+$ -N concentrations in surface soil (0–5 cm) at incubation times 0, 2 and 5 days were higher for the no-till soil than for the ploughed soil (Fig. 2 a). The  $\text{NO}_3^-$ -N concentrations did not differ between the management practices (Fig. 2 b). Soil total C and total N contents were higher in the no-till soil than in the soil of the ploughed plots, whereas the C/N ratio and soil water content did not differ between the management practices (Figs. 2 c, d, e and f). The dry bulk density of the soil was higher for the ploughed soil than for the no-till soil (Fig. 2 g).

The highest gross N transformation rates were found for  $M_{\text{Norg}}$  and  $I_{\text{NH}_4}$  (Fig. 3). Both of those process rates were higher in the no-till than in ploughed soil, as was  $R_{\text{NH}_4}$ . The relative difference of the soil treatments in  $M_{\text{Norg}}$  was 14%.

The gross rates of the other processes ( $L_{NH_4}$ ,  $O_{NH_4}$ ,  $R_{NO_3}$  and  $L_{NO_3}$ ) in contrast, were higher for ploughed soil, although  $R_{NO_3}$  was negligible for both treatments.  $L_{NH_4}$  was 19% higher for ploughed soil than in no-till soil, and  $L_{NO_3}$  of ploughed soil was almost 16-fold to that of no-till soil.

Calculated per soil area,  $M_{Norg}$  in no-till and ploughing was approximately the same, but  $I_{NH_4}$  was still clearly higher in no-till (Table 1). Differences between no-till and ploughing were notably high for  $O_{NH_4}$  and  $L_{NO_3}$  when observed per area (Table 1). If these calculations would be performed as in Ellert and Bettany (1995), so calculated per  $m^2$  in the same mass of soil in both soil management types, so in this case for lower soil layer in ploughing due to its higher bulk density ( $mg\ N\ m^{-2}\ d^{-1}$  in a soil layer of 5.00  $cm_{no-till}$  or in 4.48  $cm_{ploughing}$ ), the figure of the gross N transformation rates would look the same as in Fig. 3 but on a different scale.

## Discussion

### Process rates in the nitrogen cycle

No-till and ploughed agricultural soils showed differences in the gross transformation rates of the processes of the N cycle. Published studies that compare simultaneous multiple gross N transformation process rates in no-till and tilled soils *in situ* under similar conditions to ours are non-existent as far as we are aware. As our data is novel it opens several interesting questions for the future research.

We observed higher gross rates for mineralization and  $NH_4^+$  immobilization, but lower gross rates for  $NH_4^+$  oxidation and  $NO_3^-$  loss flux in no-till soil plots in comparison to moldboard ploughed soil plots post barley harvest. The course of our mineralization rate ( $M_{Norg}$ ) result (higher in no-till soil than in ploughed soil) is consistent with studies in other humid regions. Muruganandam *et al.* (2010) found higher gross mineralization rates in no-till than in moldboard ploughed soil (0–10 cm) in a  $^{15}N$ -label *in vitro* experiment. Gómez-Rey *et al.* (2012) observed higher gross mineralization rates after harvest in conservation till (8 years under no-till and 6 years under reduced till) than in conventionally till soil (0–5 cm) for a maize – rye-grass rotation system, the experiment also performed *in vitro* with a  $^{15}N$ -label method. Both Muruganandam *et al.* (2010) and Gómez-Rey *et al.* (2012) observed that

mineralization decreased soon (4 hours – 2 days) after the  $^{15}\text{N}$  incubation had started. Gómez-Rey *et al.* (2012) suggested that the depletion of readily available substrate probably caused the decline in the mineralization gross rate. Neugschwandtner *et al.* (2014) observed that specifically mineralizable N was higher in no-till soil than in moldboard ploughed soil (0–10 cm).

When calculated per area we found hardly any difference in the gross rates of  $M_{\text{Norg}}$  between the two practices. Thus, the lower dry bulk density of our no-till samples balanced the  $M_{\text{Norg}}$  results between the soil treatments when calculated per soil area. When mineralization is considered from the point of view of nutrient leaching risk, the result calculated per area becomes more relevant. In this respect, it seems that the soil practice method have no effect. No-till soils usually have higher dry bulk density in comparison to that of ploughed soils (Regina and Alakukku 2010; Sipilä *et al.* 2012). This probably had been the case also in our experiment, if the sampling depth would have been deeper, like 0 – 20 cm as in the experiment of Regina and Alakukku (2010). Mathematically, higher dry bulk density in no-till soil would have increased the gap in  $M_{\text{Norg}}$  between no-till and ploughing calculated per soil area. Therefore, it is relevant to discuss the  $M_{\text{Norg}}$  gross rate result further. In addition to the contribution made by the mineralization process, the available  $\text{NH}_4^+$  was increased faster in the no-till soil by the release of adsorbed  $\text{NH}_4^+$  ( $R_{\text{NH}_4}$ ).

The observed higher immobilization rate ( $I_{\text{NH}_4}$ ) in no-till agrees with Muruganandam *et al.* (2010), who found higher gross immobilization rate in no-till than in moldboard ploughed soil especially on a short  $^{15}\text{N}$  incubation time, although this observation was made in spring. Similar immobilization results have been found in South-America, where Vargas *et al.* (2005) reported in cornfields higher microbial immobilization in no-till than in a conventional till soil at 0–5 cm depth. Interestingly, Jones *et al.* (2013) used nanoSIMS technology in their study and found that N uptake by microbes can occur within minutes after its addition to soils. The higher immobilization rate ( $I_{\text{NH}_4}$ ) could be beneficial for plant N uptake in the following growing season, as potential N storages are formed in autumn. Also  $\text{NH}_4^+$  loss flux rate ( $L_{\text{NH}_4}$ ) was lower in no-till. The  $\text{NH}_4^+$  loss flux rate ( $L_{\text{NH}_4}$ ) in this experiment is practically lateral diffusion.  $\text{NH}_4^+$  is usually highly immobile in soils (Vogeler *et al.* 2011), but due to the  $^{15}\text{N}$  concentration differences between the labeled soil column and the surrounding soil, some of the  $^{15}\text{N}$  diffused to unlabeled

area. Therefore, the ecological meaning of  $L_{NH4}$  is probably negligible as this is largely related to the  $^{15}N$  label.

The gross rates related directly to  $NO_3^-$  turnover ( $O_{NH4}$ ,  $R_{NO3}$  and  $L_{NO3}$ ), were higher in the ploughed soil. Regardless of the mostly higher substrate ( $NH_4^+$ ) concentration and higher substrate production rate ( $M_{No_{org}}$ ) or higher substrate release rate ( $R_{NH4}$ ) for nitrification in no-till plots, nitrification rate ( $O_{NH4}$ ) was over twelve times lower in no-till than in ploughing. The nitrogen cycle is a complex combination of different processes with direct or indirect interactions. The most important explanation for the lower  $O_{NH4}$  in no-till soil is the enhanced immobilization of  $NH_4^+$  ( $I_{NH4}$ ). Interestingly, heterotrophic bacteria have been observed being better competitors for  $NH_4^+$  than chemolithotrophic nitrifiers (Verhagen and Laanbroek 1991). Similar to our finding, Gómez-Rey *et al.* (2012) observed higher gross nitrification rate in conventionally till soil than in conservation till (8 years under no-till and 6 years under reduced till) soil, but only for an incubation time of 4–24 hours. In contrast to our results, Muruganandam *et al.* (2010) found higher nitrification rates in no-till soils in comparison to conventional till surface soils during two days of incubation. However, they observed that after two days of incubation the gross nitrification rate decreased. There are several environmental factors that affect nitrification, and it is therefore plausible that different studies give different results for the nitrification rate. The incubation time also makes a difference to the results. In addition, the  $NO_3^-$ -N concentrations in our study were very low, thus a relatively small absolute change in  $NO_3^-$ -N concentration is expected to greatly affect the  $O_{NH4}$  result.

Nitrate is highly soluble in water, which facilitates its uptake by plants, but this also makes it prone to losses by leaching at high soil moisture levels (Porporato *et al.* 2003). Therefore, the lower  $O_{NH4}$  in no-till soil would be expected *a priori* to decrease the risk for nutrient leaching to the surrounding ecosystems. Interestingly, the ratio of the gross nitrification rate to the gross immobilization rate,  $O_{NH4}/I_{NH4}$ , is reported to be a crucial factor for potential  $NO_3^-$  leaching in arable soils, i.e. there is a positive correlation between this ratio and  $NO_3^-$  leaching (Stockdale *et al.* 2002). The  $O_{NH4}/I_{NH4}$  ratios in our soils were 0.009 and 0.183 in no-till and ploughing, respectively, which indicates a decreased risk for leaching from the no-till soil of our study.

There was no significant difference in  $NO_3^-$ -N concentrations between the soil management practices. This would be surprising if only  $O_{NH4}$  would be

considered, as this process rate was many times higher in the ploughed soil than in the no-till soil. However, in addition to the  $O_{NH_4}$ , the  $L_{NO_3}$  was much higher in the ploughed soil, so the loss flux rate of the formed and/or added  $NO_3^-$  was very high in ploughed soil of our experimental site. Thus, both  $O_{NH_4}$  and  $L_{NO_3}$  indicate higher leaching risk of  $NO_3^-$  in ploughed soil. Unlike  $L_{NH_4}$ , the  $L_{NO_3}$  can't be explained only by the label addition, since the difference in  $L_{NO_3}$  between the soil treatments was so high.

Soils with low oxygen content have an increased risk of  $N_2O$  emissions (Tietema *et al.* 2007), which is a relevant aspect of no-till as compact soil can lead to decreased soil oxygen content when the soil is wet. Several studies from different parts of the world have shown higher  $N_2O$  fluxes from no-till soils than from tilled soils during growing season (Oorts *et al.* 2007b; Dong *et al.* 2012; Hu *et al.* 2013) and cumulatively in the first 10 years after adopting no-till practice (Six *et al.* 2004). The situation of the  $N_2O$  emissions may therefore reverse when no-till is practiced for extended periods (Six *et al.* 2004). Higher  $N_2O$  emissions from no-till soil than from ploughed soil have also been observed in Jokioinen (Regina and Alakukku 2010; Sheehy *et al.* 2013). The soils studied by Sheehy *et al.* (2013) were the same as in our experiment. Our gross N transformation rate results give some interesting supplementary information regarding the  $N_2O$  emissions to reflect upon.  $NO_3^-$  is a source of  $N_2O$  (Maier 2009), and we found higher  $O_{NH_4}$  in ploughed soil than in no-till soil. However, the  $L_{NO_3}$  was much higher in ploughed soil. Because  $L_{NO_3}$  include  $NO_3^-$  leaching, one partial reason for the lower  $N_2O$  fluxes from the ploughed soil in our site could be that although the  $NO_3^-$  production rate was high, it was also leached fast from the field. Moreover, the  $N_2O$  measurements by Regina and Alakukku (2010) and Sheehy *et al.* (2013) were not taken simultaneously as our samplings, and both the  $N_2O$  flux results and the process rate results analyzed in this present study were regulated by soil conditions prior and concurrent to samplings. Therefore, the extent to which  $NO_3^-$  loss by leaching mitigated the local  $N_2O$  emissions from the ploughed soil cannot be stated with any certainty.

#### Soil properties in relation to nitrogen cycle processes

Soil N cycle process rates are influenced by the physical, chemical and biological properties, which again are affected by the conditions in the surrounding environment and by anthropogenic actions. Thus, comparisons of gross process rates of the N cycle in our soils with those of other studies

performed in different conditions will have limiting meaning. It has been found that gross N mineralization correlates positively with soil total C and total N contents (Booth *et al.* 2005). Moreover, soil N content correlates positively with gross immobilization rate (Gómez-Rey *et al.* 2012). Those findings are compatible to our results of higher total C and total N contents and higher  $M_{Norg}$  and  $I_{NH_4}$  in no-till soil. Booth *et al.* (2005) also discovered that gross N mineralization is positively correlated with soil microbial biomass, and Sipilä *et al.* (2012) observed higher microbial biomass in no-till soil than in moldboard ploughed soil in Southern Finland. Half of the study sites reported by Sipilä *et al.* (2012) located in the same site as our study. Muruganandam *et al.* (2010) suggested that the higher N transformation rates in no-till compared to moldboard ploughed soil were primarily due to higher microbial biomass rather than microbial community structure.

Soil temperature and soil moisture can significantly affect the gross N transformation rates. The gross nitrification rate in humid climate have observed correlating positively with soil moisture and negatively with soil temperature in no-till soils during a growing season (Hu *et al.* 2013). The water content did not differ between the soil practices of our study. Therefore, soil moisture does not explain the differences in gross N transformation rates found in our present study. However, we did find that water content was higher at the end of the experiment than at the beginning. Therefore, the increased soil moisture might have affected the N cycle processes at the end of the experiment by affecting microbial activity for both types of soil managements.

We found slightly higher bulk density in the ploughed soil compared to the no-till soil, which is in disagreement with the results of Regina and Alakukku (2010). However, the bulk density of the topmost soil can well be expected to be lower in no-till soil, since the organic matter has accumulated more in the surface soil layer. The conflicting results are plausible for soil samples containing relatively different amounts of the topmost layer and deeper soil layers. Regina and Alakukku (2010) took their samples from 0–20 cm depth, whereas we took our samples from 0–5 cm depth. Soil bulk density may impact upon inorganic N transformation rates and microbial communities in general (Li *et al.* 2002; Hamonts *et al.* 2013). Thus, the different sampling depths likely affected N transformation rates due to changes in bulk density.

## Concluding remarks

Our results of gross N dynamics are not straightforward from the perspective of the environmental benefits of no-till versus ploughing in agronomy. However, the factors that favored no-till were more evident. The dynamics of soil N are important with regard to the potential for N leaching.  $\text{NH}_4^+$  is considered highly immobile (Vogeler *et al.* 2011) and hence, the  $\text{NH}_4^+$  leaching risk is negligible. However,  $\text{NH}_4^+$  is a potential source for nitrification, which in turn leads to a higher risk for  $\text{NO}_3^-$  leaching. Increased immobilization of  $\text{NH}_4^+$  diminished the potential available substrate for nitrification in no-till soil after harvesting. It could be beneficial also because plant N uptake may increase in the subsequent growing season as  $\text{NH}_4^+$  is stored fast in organic matter after harvesting. The lower oxidation rate of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  was much lower in no-till soil in our short-time study. Therefore, also because of this, the no-till soil exhibit a lower risk for  $\text{NO}_3^-$  leaching after harvesting, which is supported by the lower  $O_{\text{NH}_4}/I_{\text{NH}_4}$  ratio, associated with leaching. Also the lower  $\text{NO}_3^-$  loss flux rate in no-till soil support the lower risk of  $\text{NO}_3^-$  leaching.

Studies of the process specific transformation gross rates of the N cycle in agroecosystems are limited, and different conditions affect the importance of the processes in an ecosystem. Therefore, more extensive knowledge of the topic is needed. Our study showed different process rates in the topsoil of no-till and ploughed fields in a northern climate in a short-time study. Thus, it would be interesting to determine whether there could be a logical continuum in specifically identified topsoil conditions, microbial communities, gross process rates and environmental emissions.

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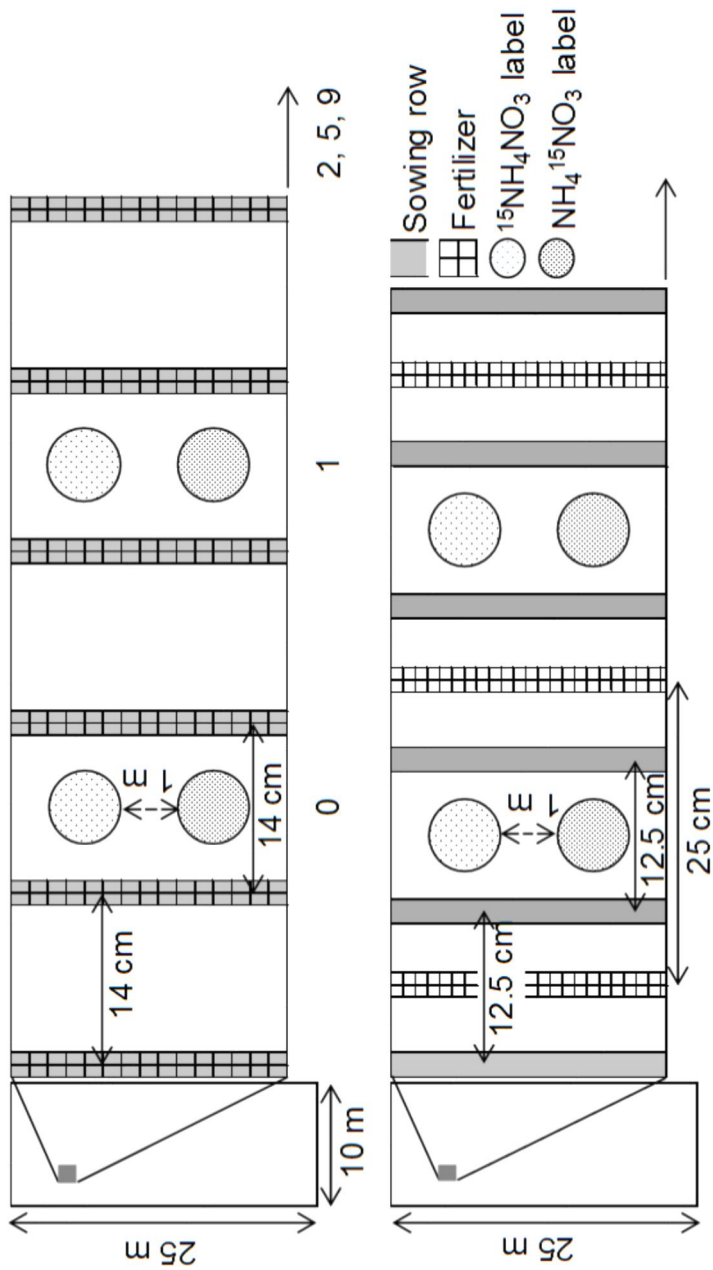
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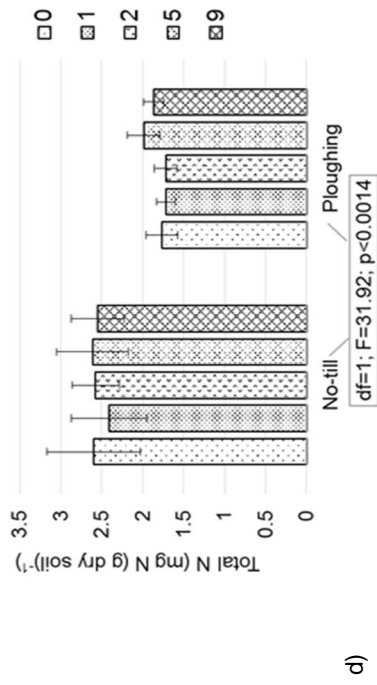
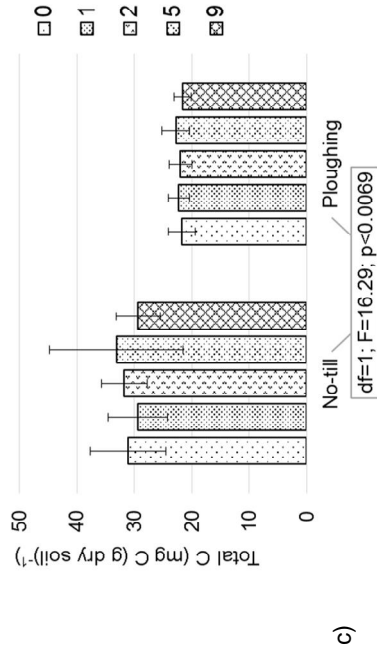
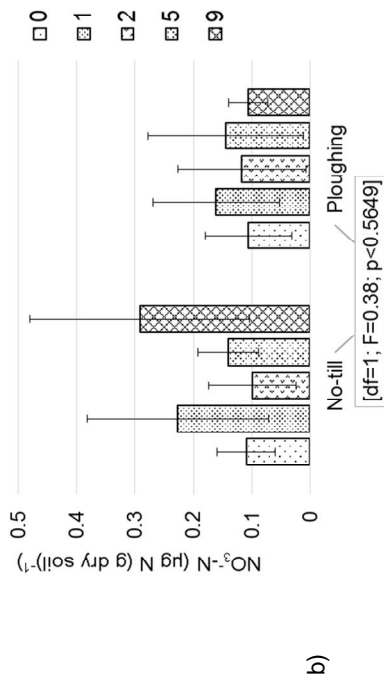
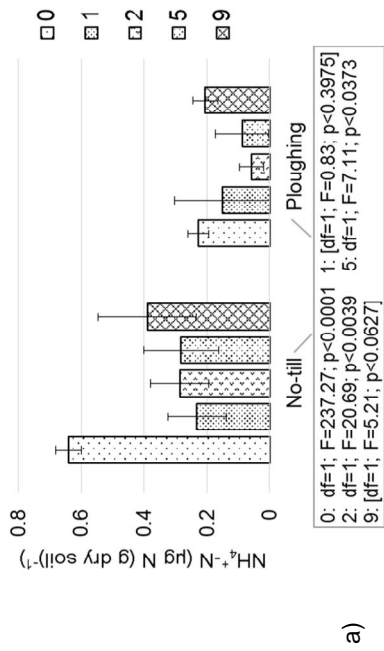
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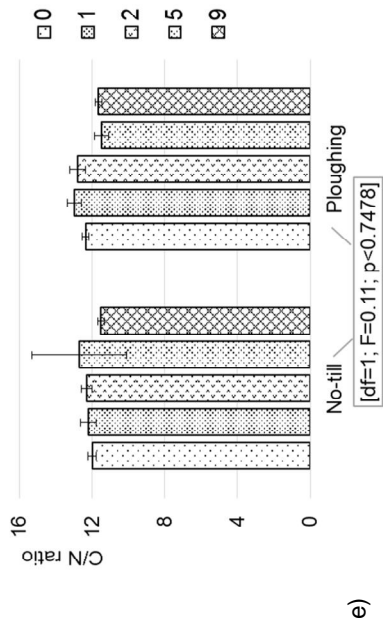
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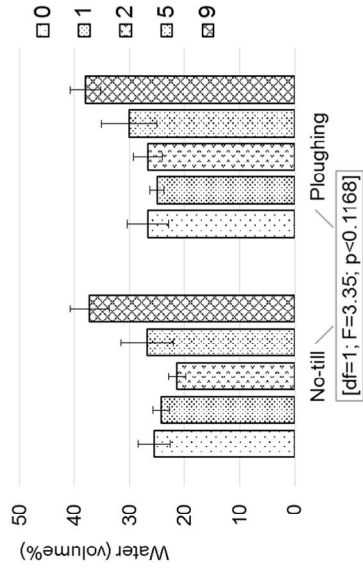


**Fig. 1** Schematic presentation of seed sowing and fertilizing in no-till (upper) and ploughing (lower) treatments, and the position of the labeling points (circles). Incubation time 0 days is on the left and incubation time 1 day is on the right. The rest of the incubation days continued similarly to the right.

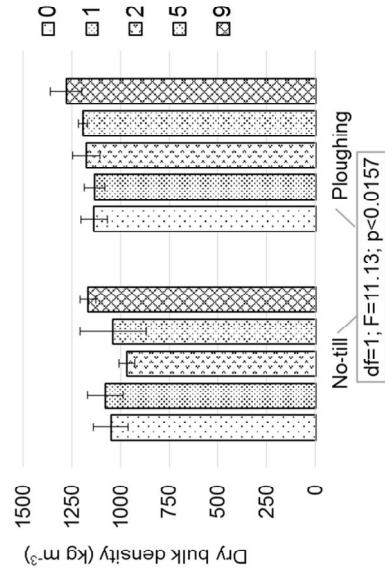




e)



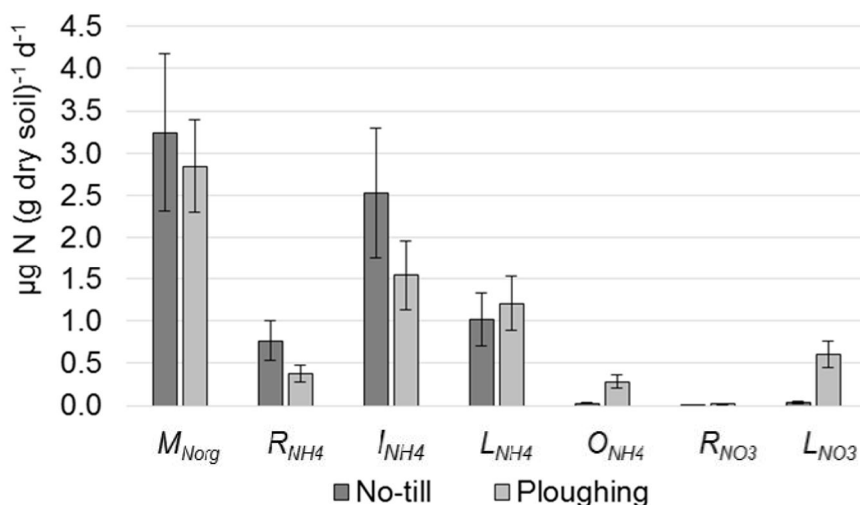
f)



g)

**Fig. 2** a) Mean values (columns)  $\pm$  SD (error bars) and statistical test results (one-way ANOVA) between no-till and ploughing of  $\text{NH}_4^+$  concentrations at different incubation times. b), c), d), e), f) and g) mean values  $\pm$  SD and one-way repeated measures ANOVA results between no-till and ploughing of  $\text{NO}_3^-$  concentrations, total C and total N, C/N ratio, soil water content and soil dry bulk density. Non-significant results are in square brackets. Different patterns of the columns: incubation times of 0, 1, 2, 5 and 9 days.





**Fig. 3** Mean quantified N transformation gross rates (columns)  $\pm$  SD (error bars) within 0–9 day incubation in 0–5 cm deep soil layer. Processes: mineralization of organic N to  $NH_4^+$  ( $M_{Norg}$ ), release of adsorbed  $NH_4^+$  ( $R_{NH4}$ ), immobilization of  $NH_4^+$  to organic N ( $I_{NH4}$ ), loss flux of  $NH_4^+$  ( $L_{NH4}$ ), oxidation of  $NH_4^+$  to  $NO_3^-$  ( $O_{NH4}$ ), release of stored  $NO_3^-$  ( $R_{NO3}$ ) and loss flux of  $NO_3^-$  ( $L_{NO3}$ ).

**Table 1** N transformation gross rates calculated as  $mg\ N\ m^{-2}\ d^{-1}$ . Processes: mineralization of organic N to  $NH_4^+$  ( $M_{Norg}$ ), release of adsorbed  $NH_4^+$  ( $R_{NH4}$ ), immobilization of  $NH_4^+$  to organic N ( $I_{NH4}$ ), loss flux of  $NH_4^+$  ( $L_{NH4}$ ), oxidation of  $NH_4^+$  to  $NO_3^-$  ( $O_{NH4}$ ), release of stored  $NO_3^-$  ( $R_{NO3}$ ) and loss flux of  $NO_3^-$  ( $L_{NO3}$ ) in 0–5 cm deep soil layer.

	No-till		Ploughing	
	Rate	SD	Rate	SD
$M_{Norg}$	134.0	38.6	131.3	25.2
$R_{NH4}$	31.8	9.7	17.7	4.7
$I_{NH4}$	104.7	31.8	71.2	19.1
$L_{NH4}$	42.1	12.8	55.8	14.9
$O_{NH4}$	1.0	0.3	13.0	3.5
$R_{NO3}$	0.0	0.0	0.8	0.2
$L_{NO3}$	1.6	0.4	28.0	7.3